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THE EFFECT OF LOW INTENSITY 2.45 GHz MICROWAVE RADIATION ON THE INFECTION OF ESCHERICHIA COLI, STRAIN B, BY T4 r_{II} PHAGE

by

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Effect of Low Intensity 2.45 Ghz Microwave Radiation on the Infection of *Escherichia coli*, Strain B, By T4 r_{II} Phage" submitted by Conrad M. B. Walker in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

The number of variables in a genetically specific phage-*Escherichia coli* system is minimized in an attempt to determine the effect of thermally insignificant, 2.45 GHz microwave radiation on the infection of an r_{II} mutant of the T4 bacteriophage to *Escherichia coli*, Strain B.

Results varying from a 90% decrease to a 70% increase in infection are obtained and several hitherto undetected variables are disclosed. Apparatus and experimental protocol are significantly modified during the course of the experiment, and improved results are obtained using radiation inside a waveguide, rather than in an antenna field.

In general, it is concluded that microwave radiation produces a change in the rate of infection of *Escherichia coli B* by phage. This change is usually a reduction in infections provided that the concentration of the phage at the time of radiation falls within specific limits.

ACKNOWLEDGEMENTS

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1. INTRODUCTION:

Towards the end of the 19th Century, when devices capable of producing electromagnetic radiation first became available, investigators began to speculate on the possible effects of such radiation on biological systems. Initially, only equipment producing electric or magnetic fields was employed; among the earliest effects noted was a flickering sensation in the presence of a varying magnetic field (17). As equipment improved, many different biological systems were subjected to electromagnetic radiation. Included among these were man, rats and dogs (18), (19) and (20). Already at this period, there was controversy as to whether non-thermal effects existed (19), (21) and (22).

During the 1930s, chemical systems (23), enzyme systems (24), (25) and a system of streptococci which was later injected into a rabbit (26) were all radiated with electromagnetic fields of various frequencies. Results were usually indeterminate in that increases or decreases in measured parameters were often recorded although there was no apparent change in experimental conditions. This naturally led to doubts as to the validity of the experiments because they were difficult to reproduce.

With the development of radar during World War II, equipment capable of producing significant power levels of V.H.F., U.H.F. and microwave radiation became available to the investigator and an ever-increasing number of biological effects were reported; again, most of them were plagued by indeterminate results, and the thermal versus athermal controversy intensified.

It was established early on that thermal effects definitely did exist, several medical studies on radar personnel revealing damage to organs such as the eye (27), the testes (28), bone marrow (29) and protein (30), (31). Some studies as to a possible cancer cure were conducted (32), (33) and there were attempts at establishing the dielectric properties of the human body (34), (35). Eye cataracts on small mammals were also reported (36), (37).

Of greatest interest here however, are the occasional studies that were made on the effects of radiation on micro-organisms. Fleming (38) reports lethal effects on Escherichia coli under radiation at fairly high power levels, though few details are given. Nyrop (39) did experiments with various bacteria and viruses, again finding a lethal effect at fairly high power. It is interesting to note that this investigator was among the first to use a pulsed source to reduce heating effects.

The above experiments were carried out in a capacitor field, rather than in an antenna or waveguide field as was achieved in this experiment. The effects are therefore due to a rapidly varying electric field rather than an electromagnetic field. At about this time, the first indisputably athermal effect was noted. This was the so-called "pearl-chain" phenomenon in which particles aggregate in long lines in the direction of the electric field. The effect has been observed with several different organisms and materials and is widely reported (40), (41), (42), (43), (44), (45).

With the use of microwave devices, including radar, communications, industrial heating and domestic cooking equipment becoming

more widespread, there arose a growing concern over the possible health hazards of radiation. The literature contains many reports of microwave-induced disease, including lenticular eye damage (46), cataracts (47), testicular damage (48), blood effects (49), (50), brain and nervous system effects (51), and effects on the unborn foetus (52). One of the most recent reviews on this subject area is contained in a book by Marha, Musil and Tuha (16).

Much of the work was done in the U.S.S.R. and incomplete reporting led to scepticism among investigators in North America and elsewhere. This is borne out by the vastly different standards of permissible radiation levels in various countries. Below are depicted some of the environmental exposure standards established in several countries, further illuminating the controversial situation.

RADIATION STANDARDS (All for the microwave frequencies 300 MHz to 300 GHz)

Czechoslovakia

Pulsed: Pro-rated on a basis of a maximum of 10 μW/cm^2 per working day (8 hours). (e.g. 20 μW/cm^2 for 4 hours etc.).

CW: Pro-rated on a basis of a maximum of 25 μW/cm^2 for an 8-hour day.

U.S.S.R.

Pulsed and CW: Maximum of 10 μW/cm^2 per working day or, if essential work is to be carried out, a maximum period of 2 hours per day at power densities of 0.01 to 0.1 mW/cm^2, and a maximum period of 15 to 20 minutes per day at power densities of 0.1 to 1.0 mW/cm^2, providing protective clothing is worn.

U.S.A.

Pulsed and CW: Maximum power density of 10 mw/cm^2 with no time restriction. For periods less than 6 minutes, an energy density of 1 mw hr/cm^2 is allowed.

Those who claim that no dangerous athermal effects exist are proponents of the less restrictive environmental standard, and vice versa. Discussions of these and other reasons for the discrepancies are contained in papers by Frey (53), Marha (54), Ingus (55), Voss (56) and Osepchuk (57).

2. OUTLINE OF PROPOSED EXPERIMENTS

THE BIOLOGICAL SYSTEM

In order to eliminate some of the undesirable factors mentioned previously, it was decided to employ a simple biological system of well-defined genotype. The system chosen was a virus-bacterium system in which the strains of virus and bacteria were maintained the same throughout the experiment, thereby minimizing genetic differences.

The particular system chosen was the phage - *Escherichia coli* (*E.coli*) system, the genotypes being rED a42, a rapid lysis mutant (r_{II}) of bacteriophage T4 obtained from Dr. R. Hodgetts, and *E.coli B*, a bacterium which is sensitive to phage a42 (12). When the phage and the bacterium are brought together the tail fibres of the phage adsorb onto the surface of the bacterium. These tail fibres are composed of a long protein chain. When adsorption has occurred, the phage injects its hereditary material into the bacterium, and the phage commences to multiply inside the host. When there are approximately 200 phage in the bacterium, the walls of the latter burst, releasing free phage into the surrounding medium. These phage then infect other bacteria and the process is repeated. This "destruction" of bacteria is known as lysis. The time course of events is approximately as shown in fig. 1. The rate of multiplication is affected by the temperature of the system. The time from infection to bursting of the bacterium follows a distribution of the form indicated in fig. 2.

NB. THESE CURVES ARE FOR ILLUSTRATIVE PURPOSE ONLY AND ARE MODIFIED FROM GRAPHS AND DESCRIPTIONS IN (11).

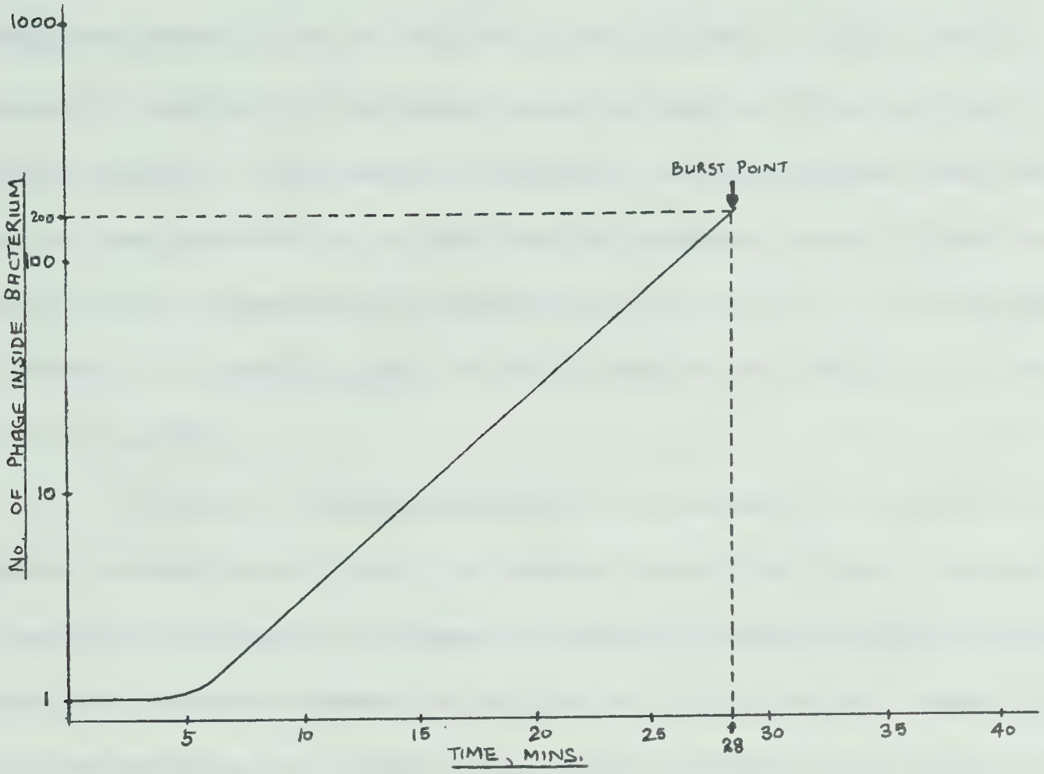


FIG. 1 TIME COURSE OF PHAGE MULTIPLICATION AT 37°C

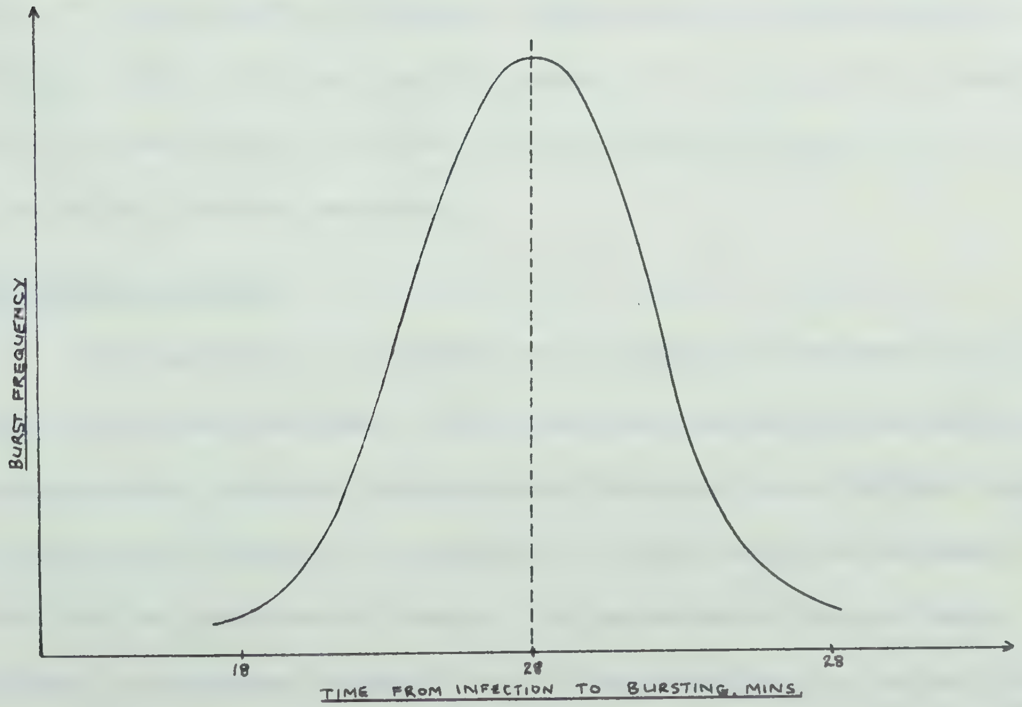


FIG. 2 DISTRIBUTION OF BURST FREQUENCY AGAINST TIME

One other factor to be considered is the possibility of premature bursting due to rupture of the bacterial wall, perhaps caused by passing the bacteria through a small orifice such as a 0.1 ml pipette. After about 10 minutes, the wall becomes very delicate and is thus susceptible to this kind of rupture. As will later be seen in the discussion of the experimental procedure, this premature bursting could easily cause incorrect results and should therefore be guarded against.

When $\sim 10^7$ E.coli B bacteria are spread on an agar plate, they form a greyish-brown "lawn", on the surface of the plate. However, when a bacterium is lysed, it becomes colourless, since the phage which have taken its place are themselves colourless. Thus, when a number of infected bacteria are plated along with uninfected ones, clear spots will appear whenever an infection has occurred, the spots being the result of a large number of bacteria being lysed as more phage are produced. These spots are called plagues. The size of the plagues depends on temperature and other factors such as the age of the cultures and the actual strains employed.

EXPERIMENTAL OUTLINE

The purpose of this work was to employ microwaves in an attempt to perturb the phage - host cell interaction in the early stages. This interaction involves adsorption of the phage onto the host cell and injection of DNA into the latter. In this case, use was made of a control and an experimental sample, the experimental sample being subjected to microwave radiation while the infections of bacteria by

phage were taking place. After radiation, both control and experimental samples were appropriately diluted, and the number of phage adsorbed to the bacteria was measured. A comparison of the number of plagues on each plate was then an indication of the effectiveness of the radiation on adsorption and/or injection. Detailed procedures are given in chapter 7.

Two different methods of radiation were employed.

(i) Radiation of the sample in the near field of microwave horn antenna.

(ii) Radiation of the sample in a waveguide.

Power levels of the same order were employed in both cases, and the frequency was always 2450 ± 30 MHz. The power level was maintained in the region of 5 - 10 mW/cm^2, a level at which insignificant heating occurs over the time period in question; this is further discussed in chapter 6. Many difficulties exist in establishing the actual power level. Some of these are discussed in chapter 4 and Appendix 1. The literature contains several references to this problem, (14), (1), (15), (16).

A few experiments were carried out using continuous wave (cw.) radiation, but for the most part, pulsed radiation was employed. During initial experiments, there was some variation in pulse lengths and repetition rates, but in later experiments, these parameters were maintained constant.

3. CROSS REFERENCING METHODS EMPLOYED

Over the period during which experimental work was done, a number of experiments were performed. The experimental protocol was modified on several occasions, as was the electronic apparatus, changes being made in accord with results obtained. Thus, the experiments have been numbered in chronological order, so that the equipment and protocol employed on each occasion can easily be referred to the correct experiments.

Some experiments had to be rejected for one reason or another and although the results of such experiments are omitted, their numbers are still used in descriptive sections.

As the experimental work proceeded, various hitherto unconsidered variables made their appearance. It is therefore reasonable to suppose that some of the anomalous results can be attributed to as yet unsuspected variables whose nature could only be determined by further experiments. The presentation of the results in chronological order, the actual dates, and as far as possible the time of day that the experiments were performed may thus prove of value in establishing a further variable. On looking back, it is realized that there are insufficient data concerning each experiment, and a complete analysis of the effect to be described should include reference to ambient atmospheric data, such as magnetic field,

humidity, temperature, and pressure, as well as a careful record of the actual time course of events during the experiment and the small changes that these were subjected to. If these data were available, it might be possible to establish further variables which are of importance. Chapter 10 deals with some of the variables which might have proved useful, and which should be recorded in later experiments.

4. ANTENNA AND WAVEGUIDE RADIATION SYSTEMS

INTRODUCTION

As was mentioned above, experiments 1 to 15 were performed in the near field of a microwave horn, while experiments 16 to 25 were performed in a waveguide.

THE MICROWAVE HORN ANTENNA

The horn had dimensions as indicated in Fig. 3.

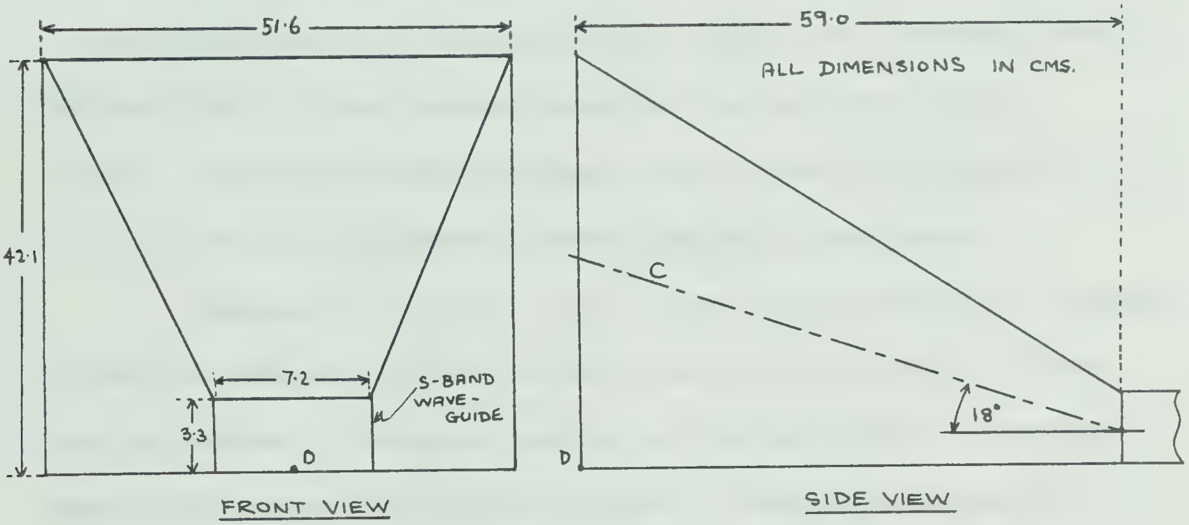


FIG. 3 MICROWAVE HORN ANTENNA

The frequency of operation was 2450±30 Mhz., the power level being measured off a directional coupler placed shortly before the horn. Point D on the diagram (Fig. 3) refers to the datum point relative to which all azimuth measurements were made. A line C along which the power density was a maximum was established, and measurements were made in the plane of this line.

The horn was placed inside a copper-screeened room, the screening being internally lined with microwave absorbent material,

known as "Eccosorb". The associated electronic apparatus was outside the screened room, feed being through a well screened hole in the wall of the room.

All power density measurements were made using a Narda # 8110 power density probe on the 0 - 2mW/cm^2 and 0 - 20mW/cm^2 ranges (10). This meter employs an evaporated thin-film crossed dipole antenna and detector element. It is therefore independent of field polarization. Calibration is ± 1dB at two frequency bands, 2450 Mhz. and 915 Mhz. Maximum deviation across these bands is ± 0.1dB. The electrothermic elements have a long time constant (0.5 to 0.75secs.) to prevent damage from high peak powers.

Because of the small size of the antenna employed, coupling to the electromagnetic field is small and so perturbation of this field is minimal. The probe handle is situated behind the antenna, thereby minimizing its perturbing effect. Careful shielding and filtering of the cable connecting the probes to the meter ensures that there is no RF pickup on this cable. Different probes are used for different power density ranges. In these respects, the meter represents a considerable advance over those previously available.

Measurements were repeated several times under different conditions, these being:

(i) Metal support holding the probe in position

(ii) Non-Metal support holding probe in position.

(iii) Both of the above with and without a representative biological sample. A sample consisted of 2ml of growth medium

(Luria broth) in a glass test tube supported in a string - suspended perspex rack at 60cms. from D on the maximum power density line.

The results obtained from these tests are presented in tables 1,2, and 3 and in figs. 4 and 5.

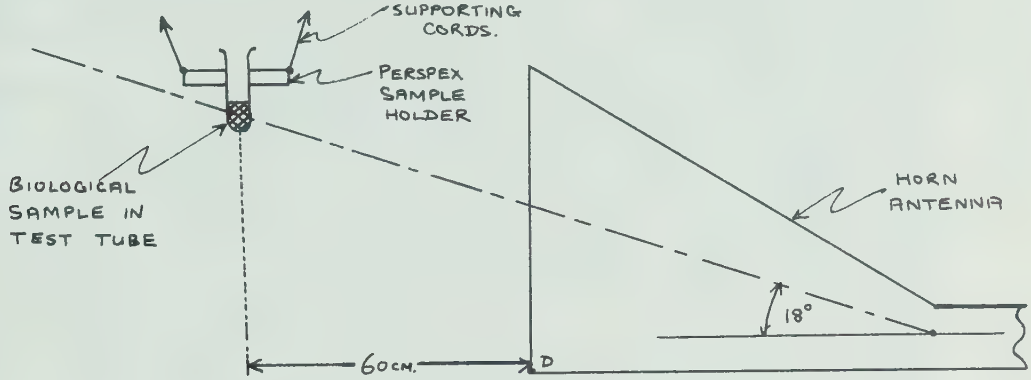


FIG. 4 LOCATION OF SAMPLE - SIDE VIEW

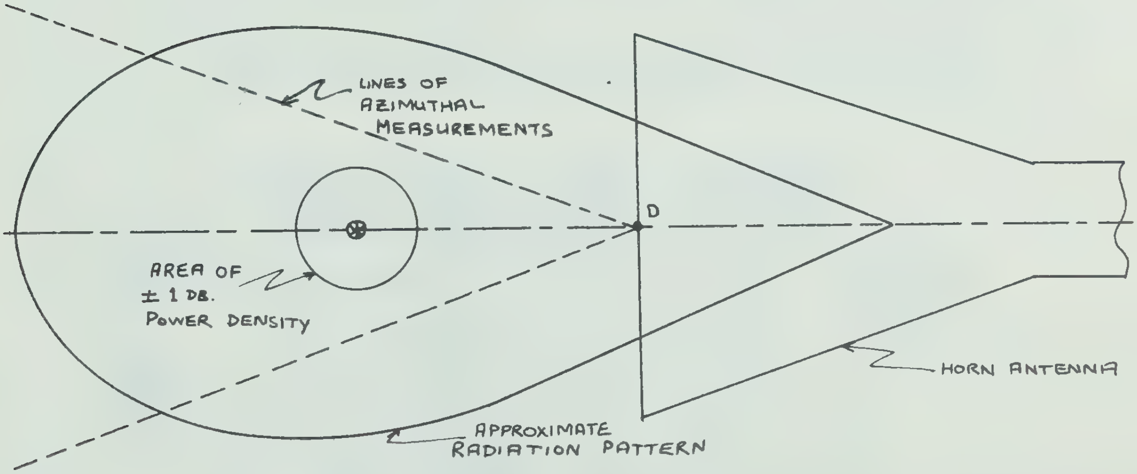


FIG. 5 LOCATION OF SAMPLE - TOP VIEW

MEASUREMENTS AT 60 CM ON THE 0° LINE WITH A POWER INPUT OF 2WATTS.

POWER DENSITY IN MW/CM²	1·80	1·90	1·60	1·58	0·61	0·38
HEIGHT ABOVE D CMS.	47	37	28	20	11	0

TABLE 1. POWER DENSITY VARIATION WITH HEIGHT

ALL POWER DENSITIES IN MW/CM²

POWER INPUT TO ANTENNA IS 2WATTS.

CARRIER FREQUENCY IS 2460MHz

ANGLE FROM 0° WITH L.H.S. +VE / DISTANCE FROM HORN, CMS	20	30	40	50	60	70	80	90
-25°	3.0	2.2	1.2	0.97	0.68	0.35	0.21	0.10
-20°	3.7	2.7	2.0	1.22	0.91	0.58	0.47	0.22
-15°	4.0	3.6	2.6	1.61	0.93	0.73	0.60	0.55
-10°	4.2	4.0	2.8	1.71	1.21	0.86	0.73	0.59
-5°	4.2	3.9	2.6	1.90	1.59	1.15	0.90	0.59
0°	4.2	3.9	2.6	1.90	1.80	1.15	0.85	0.55
+5°	4.0	3.6	2.2	1.90	1.79	1.10	0.80	0.68
+10°	3.6	3.1	2.2	1.64	1.41	0.95	0.62	0.68
+15°	3.5	3.0	2.1	1.59	1.19	0.70	0.60	0.46
+20°	3.1	2.8	1.9	1.30	0.93	0.59	0.48	0.32
+25°	2.8	2.2	1.2	0.98	0.75	0.38	0.25	0.18

TABLE 2. ANTENNA POWER DENSITY MEASUREMENTS

POWER INPUT TO ANTENNA IS 4.8WATTS AVERAGE.

CARRIER FREQUENCY IS 2455 MHz

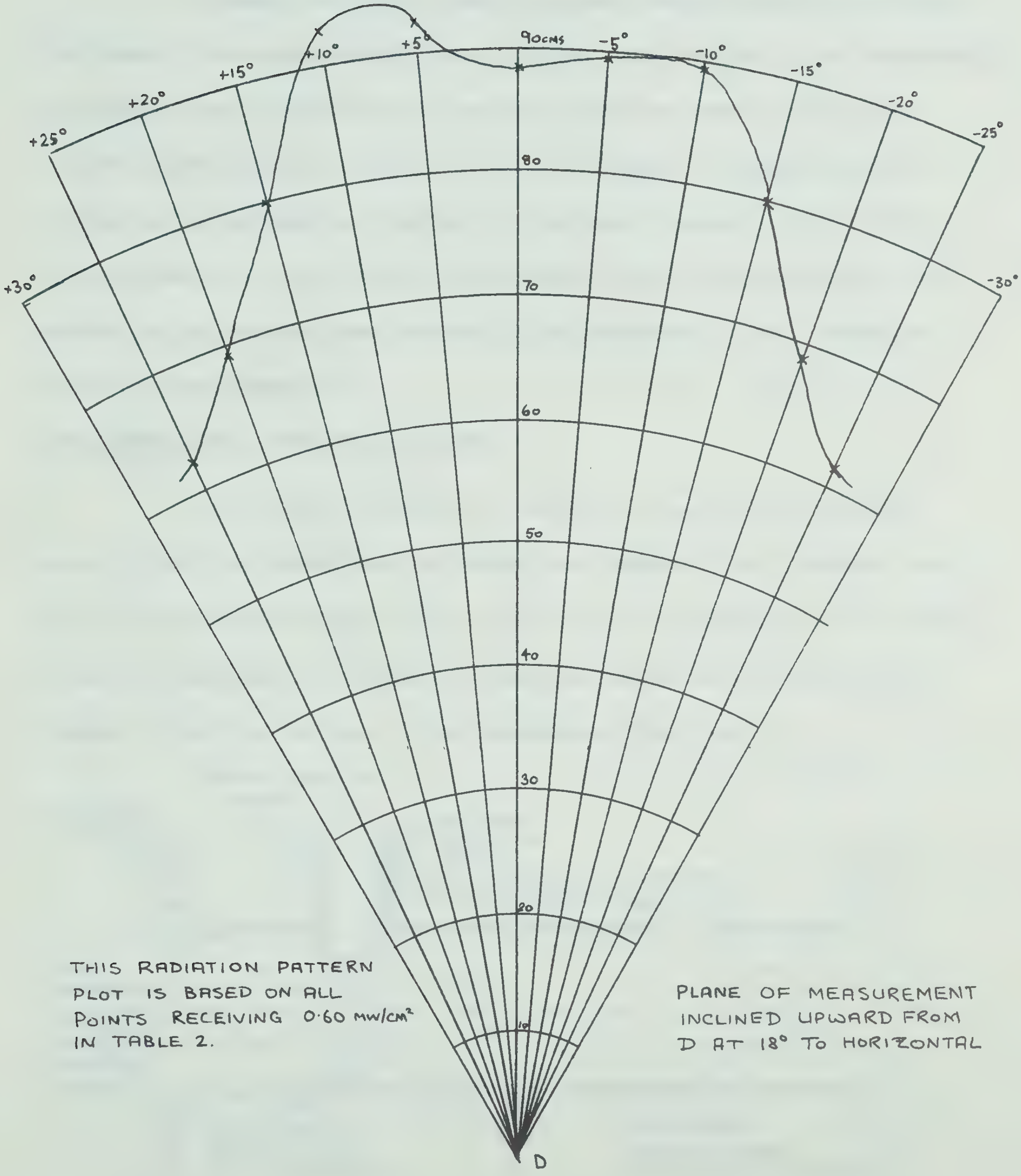
WHETHER TUBE PRESENT OR NOT	TYPE OF SUPPORT EMPLOYED	POWER DENSITY IN MW/CM²	DISTANCE FROM APERTURE, CMS.
NO	METAL	9.2	60
NO	METAL	6.0	80
YES	METAL	6.0	80
YES	METAL	6.0	60
NO	NON-METAL	8.0	60
YES	NON-METAL	5.0	60

"TUBE" REFERS TO TEST TUBE HOLDING SAMPLE

TABLE 3. EFFECT OF DIFFRENT SUPPORTS

NOTES:

1) Table 1 was used to determine the plane of maximum radiation, so that table 2 could be plotted.
2) Using table 2, an area where the power density did not vary by more than ± 1dB was delineated, and the sample was confined to this area.
3) Table 3 shows how various supports, and the biological samples affected the power density. In actual experiments, a non-metal support was utilized.
4) Figure 5a is a radiation-pattern plot of the antenna as obtained from table 2.



THIS RADIATION PATTERN PLOT IS BASED ON ALL POINTS RECEIVING 0·60 mw/cm² IN TABLE 2.

PLANE OF MEASUREMENT INCLINED UPWARD FROM D AT 18° TO HORIZONTAL

FIG. 5a RADIATION PATTERN PLOT OF ANTENNA

It should be noted that the power density with and without the sample undergoes considerable change, and further it is reasonable to suppose that the measuring process produces some perturbation in the power density as well. For this reason, the power density is not given an absolute value, but is merely stated to be within a certain range. Since we are in the near field of the antenna, the situation is further complicated by the presence of the induction field. See Appendix 2 .

THE WAVEGUIDE RADIATION SYSTEM

This simply consisted of a piece of waveguide through which a hole has been made to enable the sample to be inserted into the electromagnetic field. The open end of the waveguide was terminated in a matched water load. Power was measured just before the sample - holding section of the waveguide. (See fig. 6)

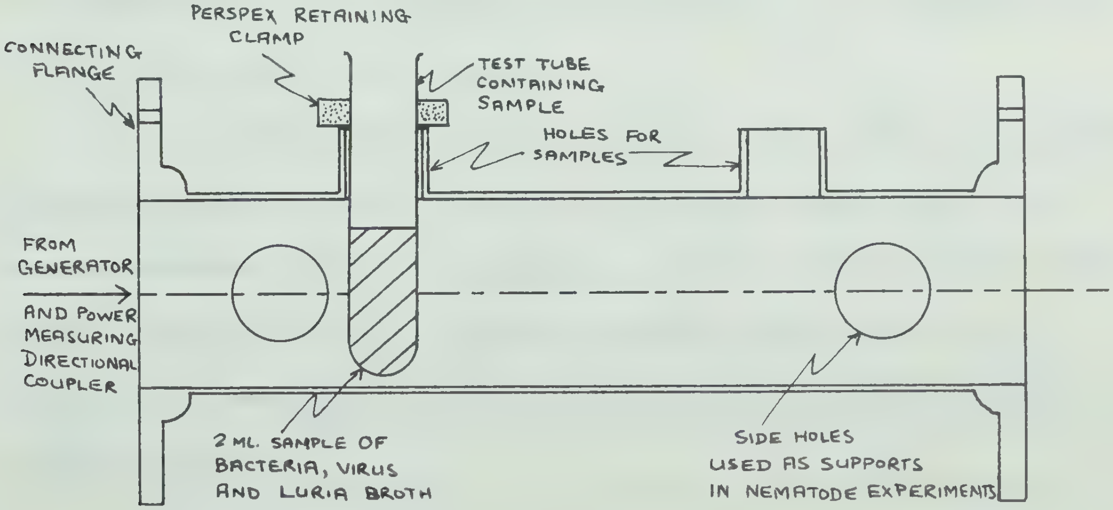


FIG. 6 CROSS-SECTION OF WAVEGUIDE SAMPLE HOLDER

With this configuration it is difficult to obtain a direct measure of the power density in the waveguide, but various deductive procedures are available.

(i) Knowing the total power in the guide, and the cross-sectional area of the guide, it is possible to calculate the average power density in the guide. This is not necessarily accurate, since the power is not evenly distributed across the waveguide. Further, the effect of the sample is not accurately known. This problem is further discussed in Appendix 3 .

(ii) By applying a higher power level to the waveguide section and measuring the temperature rise of the sample in a given time, it is possible to calculate the power absorbed by the sample. The required input power level to maintain a given power at the sample can then be calculated from this. Some of the problems involved in measuring the temperature rise are discussed in section 11 and Appendix 4 details the calculations required.

In experiments 16 to 25, the power imput level was determined by multiplying the power from the horn antenna experiments by the cross-sectional area of the waveguide. Thus, in these latter experiments, the power level is probably somewhat higher than in the earlier one, and again, absolute values cannot be quoted, only a range of values.

5. MICROWAVE GENERATION AND RADIATION APPARATUS

The three main steps in the evolution of the microwave irradiation apparatus are illustrated in fig. 7. In its original form, wavelength was measured by means of the slotted line, and waveform measurements were taken directly from the "modulation monitor" terminals on the microwave power generator. The standing wave ratio was adjusted to about 1.06:1 by means of the tuner. Since the power meter has a full scale deflection of only 10mW, 30dB attenuation was introduced to enable power measurements up to 10W to be made. It was found that the best pulsed operation of the microwave power generator was obtained by feeding pulses into the "square wave modulations" terminal and adjusting the tuning and coupling controls for the desired waveform. Operation with the "pulse input" was rather unsatisfactory.

In an attempt to remove some of the 60 cycle mains modulation observed on the oscilloscope, the modification indicated in fig. 7 b was made. This also enabled the signal to be monitored closer to the antenna. To allow more precise measurement of frequency, a wavemeter was also installed at this stage.

After 15 experiments had been performed using the above system, it was decided that the radiation apparatus could be improved by placing the test sample in the waveguide. This modification is indicated in fig. 7 c . Since the insertion of the sample has considerable effect on the VSWR, it was found necessary to insert an isolator between the generator and the waveguide system, thereby

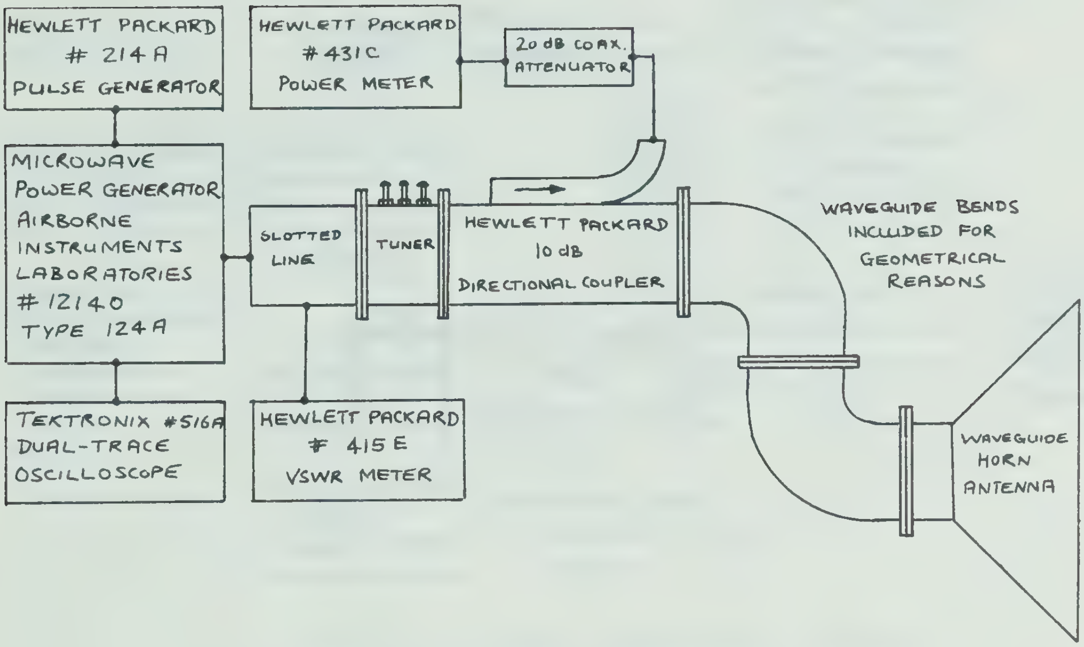


FIG. 7a. FIRST RADIATION SYSTEM

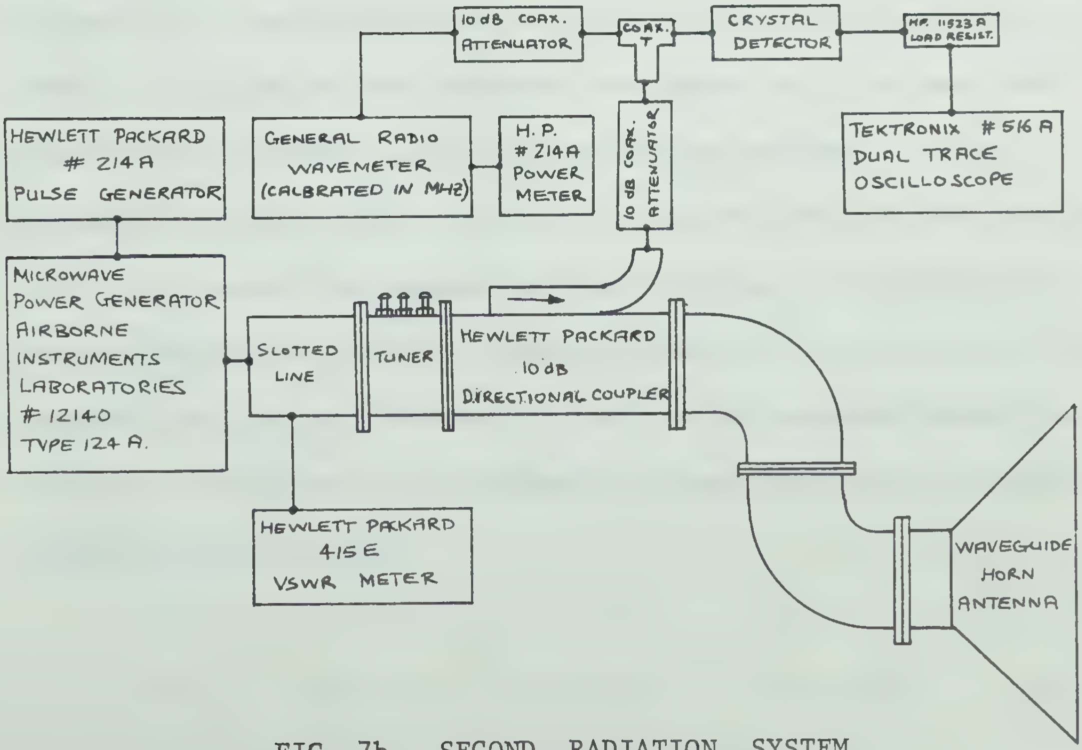


FIG. 7b. SECOND RADIATION SYSTEM

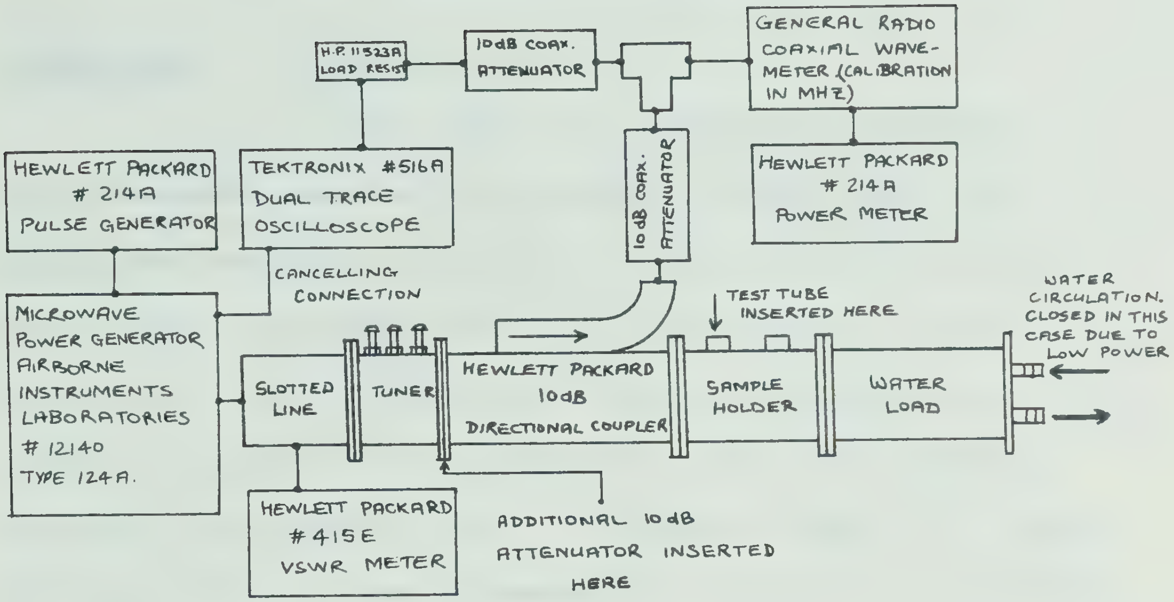


FIG. 7c. THIRD RADIATION SYSTEM

preventing power fluctuations in the oscillator as samples were inserted and removed. Further, since the oscillator only functioned satisfactorily in the output power range of 1 to 5 watts, and since a power level of only 160 mW was required, see Appendix 3, it was found necessary to introduce the 10 dB attenuator as shown in fig. 7c. In an attempt to further reduce the 60 cycle interference on the oscilloscope trace, the cancelling connection shown in fig. 7c was added, this being a connection from the modulation monitor on the generator to channel 2 on the oscilloscope. By putting the required signal on channel 1 and adding the two signals, it was found that the interference could be cancelled.

6. TEMPERATURE RISE EXPERIMENTS

INTRODUCTION

There were some doubts as to whether the experiments herein described were truly non-thermal, or whether some small temperature rise could be detected. It should here be stated that even if there was a temperature rise, it would have to be quite considerable before it became significant in this experiment. In general, it is agreed that a temperature rise of 10°C produces a doubling in the metabolic rate of a biological system (8). However, the phage-bacteria system is somewhat more sensitive to temperature, there being a doubling for every 5°C of temperature change over the normal range. The effect here described is often a decrease in infections of bacteria by phage, however, whereas an increase in temperature would be expected to increase the rate of infection (9); if anything, the temperature of the sample would be increased by the action of the microwave radiation.

A further factor rendering temperature rise unlikely is the small size of the sample, which is conducive to rapid cooling. Some calculations on the possible magnitude of the temperature rise are given in Appendix 4.

METHODS AND APPARATUS

In addition to the theoretical speculation above, it was decided to try to measure the temperature rise of the sample. Initial experiments employed a thermocouple and associated bridge (Leeds and Northrup 8632-2 temperature potentiometer). This bridge is calibrated to 1°C and can be read to perhaps 0.5°C., and so all that

can be expected from this experiment is an order of magnitude of the quantity desired.

Two 2ml samples of Luria broth were employed. When these had attained room temperature, one was placed in the waveguide (or antenna field) and the other alongside the waveguide, as a control. Their temperatures were carefully measured before and after 10 minute radiation periods. The results are shown in table (4).

In order to improve the accuracy of these measurements, and at the same time to produce a biologically useful instrument, development was commenced on an electronic thermometer capable of measuring temperature differences of the order of 0.01°C. This instrument employs a transistor as the sensing element, making use of the fact that 1°C temperature change produces a 2mV change in the transistor junction voltage. By comparing this voltage with that of another transistor held at a fixed temperature, it is possible to measure temperature change to a resolution limited by the stability of the electronic circuitry. See Appendix 5.

RESULTS

	CONTROL 1	EXPERIMENT 1	CONTROL 2	EXPERIMENT 2
TEMPERATURE BEFORE RADIATION	24·5°C	24·0°C	23·6°C	23·3°C
TEMPERATURE AFTER RADIATION	23·2°C	24·2°C	23·3°C	23·8°C

TABLE 4. RESULTS USING THE THERMOCOUPLE AND BRIDGE

NOTES:
(i) Average power density = 6.5 mW/cm^2
(ii) On/off ratio = 105/30μsecs.
(iii) Radiation time = 10 minutes
(iv) Frequency = 2455 Mhz.

	#1	#2
TEMPERATURE DIFF. BEFORE RADIATION	0°C	0°C
TEMPERATURE DIFF. AFTER RADIATION	1·4°C	1·4°C

TABLE 5. RESULTS USING THE ELECTRONIC THERMOMETER.

NOTES:
(i) Average power density = 6.5 mW/cm^2
(ii) On/off ratio = 105/30μsecs.
(iii) Radiation time = 10 minutes.
(iv) Carrier frequency = 2458 Mhz.
(v) Temperature relative to initial datum
(vi) #1 and #2 represent separate experiments.

CONCLUSION

From the thermocouple experiments, it appears that the controls had not yet stabilised to room temperature, as they exhibited a small temperature drop over the 10 minute radiation period. On the other hand, the experimental samples rose in temperature, due presumably to the microwave radiation, and at the end of 10 minutes, the temperature differences were 1.0^{0}C and 0.5^{0}C respectively.

From the electronic thermometer experiment, we conclude that the temperature rise is approximately 1.4^{0}C. It should be pointed out that the two probes (thermocouple and transistor) are of very different composition and so are likely to have differing effects on the resultant temperature measurements.

These two experiments lead to the conclusion that the temperature rise is of the order of 1^{0}C and so, as previously explained, any effects noted can be considered to be non-thermal.

7. EXPERIMENTAL DETAILS

As previously mentioned, the details of the method in which experiments were performed were modified from time to time to correct for problems which had arisen.

PLAQUE ASSAY TECHNIQUE

The concentration of the phage stock was assayed from time to time throughout the course of the experiment (see graph 1 under chapter 9). Experiment 3 on the other hand was performed in the microwave power laboratory, firstly to determine the strength of the virus to be used and secondly to ensure that no untoward environmental effects existed. The following description of this experiment is typical of other titre experiments which were performed from time to time in order to keep check of the virus concentration. The only differences were in the number and size of the dilutions performed. Microwave radiation is not used in a titre experiment.

In order to titre a stock of phage, one assumes some concentration of virus, then, using this value, devises a dilution series which will result in a few thousand phage per millilitre. By plating 0.1 millilitre of this final solution, a few hundred plagues will appear on the plate, and can easily be counted. Working back from this value, the actual initial concentration can be calculated. For a typical dilution series, see fig. 8.

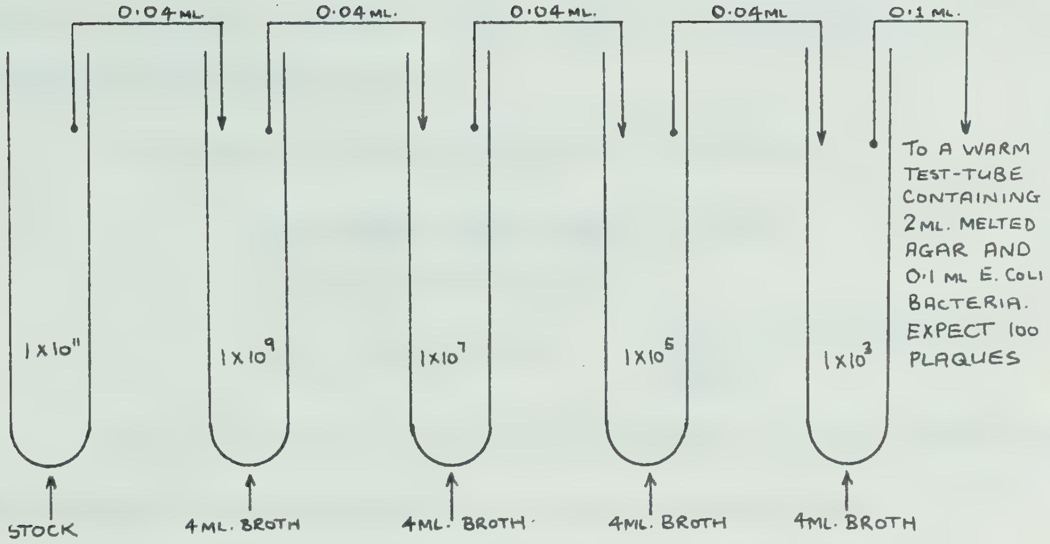


FIG. 8 TYPICAL DILUTION SERIES

There is usually about 4ml. total in each tube, the largest single dilution being 1/100, as indicated. The method of plating is as follows:

2ml. of top layer, slightly diluted agar, is melted and poured into a test tube. To this is added 0.1ml of indicator bacteria, in this case E.coli B as this is sensitive to the phage. The required quantity of phage (in this case 0.1ml) is added to this mixture and the whole, after vigorous shaking, is poured onto a plate. A plate is a 3" diameter plastic petri dish into which agar to a depth of approximately 1/8" has previously been poured and allowed to harden. The mixture is then allowed to harden for 15 minutes and then the plate is placed in an incubator for about 12 hours. The bacteria grow on the nutrient contained in the agar, and produce the previously mentioned greyish - brown background; at each point where a phage is present, lysis occurs and a clear spot results.

[illegible]

[illegible]

[illegible]

[illegible]

A count of the clear spots is thus a measure of the number of phage contained in the 0.1ml sample.

The total dilution in this case is given by:

$$\underbrace{\frac{1}{100} \times \frac{1}{100} \times \frac{1}{100} \times \frac{1}{100}}_{\text{Test tube dilutions.}} \times \underset{\substack{\uparrow \\ \text{Result of taking 0.1ml.} \\ \text{sample.}}}{\frac{1}{10}} = 10^{-9}$$

Thus, if say 96 plaques were obtained, we could calculate the original concentration in one of the following ways:

(i) Concentration x dilution = no. of plaques.

$$\text{concentration} = \frac{96}{10^{-9}} = 9.6 \times 10^{10}$$

(ii) Assumed concentration = $1 \times 10''$

Expected no. of plaques = 100

$$\text{actual concentration} = \frac{96}{100} \times 10'' = 9.6 \times 10^{10}$$

With slight variations, all the plating in the following experiments was carried out in this manner.

RADIATION EXPERIMENTS

Each radiation experiment has certain common factors, and these will be described first.

A logarithmic culture of E. coli B is grown from an overnight culture. An overnight culture consists of 0.1mls. of bacteria solution taken from a refrigerated culture, diluted into 10 ml. of broth and shaken for 18 hrs. (usually overnight) at 80 r.p.m. The special culture is a 1/20 dilution of the overnight culture which

is shaken for 1 hour at 120 r.p.m. This ensures that the bacteria are in logarithmic phase for each experiment, this phase being the one in which they are most receptive to infection. Two 2 ml. samples of this culture are then prepared, one for use as a control and the other as the test sample. The test sample is now placed in the field (be it antenna or waveguide) and the control sample is placed nearby, but out of the field. The field is then switched on and 0.1 ml of an appropriately diluted phage stock is added to both the control and test samples. Under normal experimental conditions, the bacterial culture has a concentration of about 2 x 10^8/ml., and the phage were added at a concentration of about 1/10 phage/bacterium. The test sample is left in the field for ten minutes as timed by a stopwatch, and, during this ten minutes, a certain number of infections take place.

To determine whether or not the microwaves had an effect on adsorption, the number of infective centres in central and radiated samples were compared. It is necessary to remove any free phage, since they would later infect the indicator bacteria and cause an erroneous plague count.

This problem of removing the phage can be approached in several different ways and it is these different methods that constitute most of the changes in experimental protocol.

REMOVAL OF UNADSORBED PHAGE:

(1) SCAVENGING BACTERIA. The first method, and that employed in experiments 5 through 19, was removal of phage by means of what will be termed a scavenging bacterium. This bacterium is a different strain of E.coli, known as E.coli K12(λ). It absorbs the free phage but does not burst, thereby effectively removing these free phage from the system. The K12(λ) has no other effect on the system.

Thus, in order to stop the infections at the end of the radiation period, a large excess of K12(λ) (about 0.5 ml of overnight culture) is added to both the control and test samples. The mixture is now allowed to stand a further 10 minutes, in order that the phage may be thoroughly scavenged. Of course, a certain number of phage - E.coli B infections occur in this time but this number will be similar in both the control and test samples, and so will not affect the final difference between these two samples.

One problem which is encountered in this protocol is that of premature bursting. Intracellular phage may begin to appear between 10 and 15 minutes following adsorption. Occasional lysis of cells at any time after this will liberate mature phage progeny, which will be confined with infective centres. This problem was only fully solved in experiment 20, although from experiment 12 onwards, immersion in iced water was used immediately after the 10 minutes scavenging period in order to slow down the bursting process. The final solution as

adopted in experiment 20 was to add 0.02 ml. of potassium cyanide (KCN) to the samples just before radiation and again just before scavenging. The KCN does not prevent adsorption of phage to either E.coli B or K12(λ), but it does prevent the phage from growing inside the bacteria. Thus any premature bursting will not result in liberation of mature phage progeny. Even at the above concentration, there are not many more K12(λ) bacteria present than there are virus, and its effectiveness is thus limited. This problem was overcome from experiment 14 onwards.

After the scavenging period is over, the two samples are independently diluted on ice to about 2000 virus per millitre before plating 0.1 mls. in the manner previously described. Usually, each sample was plated onto 4 different plates and a mean plaque count obtained.

(2) ANTI-PHAGE SERUM. A simpler method for removing the unadsorbed phage is by the use of anti-T4 serum. At the concentration of anti-serum used here, the number of phage was reduced from 10^7 phage/ml to 10^3 phage/ml in about five minutes. The dilutions were again performed in the presence of KCN and on ice to rule out premature bursting. By using methods 1 and 2 together, it was possible to get two results which should have been complementary, i.e. adding the two control values should give the same count as adding the two test values. The total obtained should agree with a simultaneously conducted titre experiment. This goal

was not achieved in experiments 21 to 24, partly, it was felt, because of the logistic problem of doing all the necessary operations in the short time required (to prevent premature bursting). For a more complete description of the combined scavenging method, see note 10 under table 8, in chapter 9.

REMOVAL OF INFECTIVE CENTRES

CHLOROFORM TREATMENT. A second method of measuring the absorption, complementary to that of determing the no. of infective centres by removal of unadsorbed phage, is to remove all the infective centres. This is accomplished by addition of 0.1 ml of chloroform prior to the end of the eclipse period (the period prior to which no mature intracellular phage are present). Immediately after diluting the infective centres to end adsorption, chloroform was added to the solution. The chloroform has no effect on the free unadsorbed phage but immediately kills all the infective centres present. Thus, on plating, only those phage which have failed to produce infections will react with the indicator bacteria. Of course after adding the choloroform, the usual dilution to a concentration of a few thousand is performed, in order to get countable numbers of plaques. Since in this experiment it is the unadsorbed phage which are counted, any result obtained should be the converse of those obtained by measuring the infective centres. Experiments 21 to 25 used this method, 21 to 24 being in conjunction with the anti-phage serum method.

8. PHAGE AND BACTERIA MORTALITY EXPERIMENTS

INTRODUCTION

In order to check the possibility of the radiation actually destroying the biological material, several experiments were performed such mortality experiments are necessary, since if either the bacteria or the virus were being destroyed by radiation, a change in plaque counts would be apparent. It would then be incorrect to attribute the observed change to a disruption of the infection process.

METHODS

In an attempt to see whether or not irradition had any effect on free phage or bacteria, the following experiment was performed: Phage, at the concentration used in the radiation experiments, was added to two samples of Luria broth, one being placed in the radiation field for ten minutes and the other being used as a control. Both were then diluted to plating strength and assayed for phage. Any damage to the phage would be reflected in the subsequent plaque counts, but as the results show, these are very similar.

One final experiment was performed which likewise indicated that phage and bacteria are not irreversibly affected by microwave radiation. Experiment 4 was performed exactly as in method 1 above except that the K12(λ) was not added. If no mortality had occurred, then there should be no appreciable difference between the test and the sample counts. The results presented below suggest that this was indeed the case.

On several occasions, samples of bacteria were radiated for 10 minutes and plated, but in all cases they appeared to grow normally.

This is in keeping with the results of an experiment to test the effect of microwaves on E.coli B6 as described in (7).

RESULTS

See table 6.

CONCLUSION

As can be seen from the results talbe, the change between individual results, and even between the means, is random and fairly small. It may thus be concluded that the radiation does not cause any significant damage to the phage or bacteria, though more extensive experiments are indicated.

RESULTS

	#1	#2	#3	#4	TOTAL	MEAN	CHANGE %AGE
CONTROL 21	95	105	–	–	200	100	-3.5
EXPERIMENT 21	84	109	–	–	193	96.5	-3.5
CONTROL 4	54	56	62	–	172	57.3	0
EXPERIMENT 4	59	57	65	48	229	57.3	0

TABLE 6. BACTERIA AND VIRUS MORTALITY EXPERIMENTS

NOTES:

1) "Control" refers to the sample that was not radiated, while "Experiment" refers to the one that was.
2) "a", "b" and "c" etc. refer to experiments performed on the same day.
3) "Change" refers to the percentage change relative to the control, + being an increase and - a decrease.
4) "#1 through #4" represent different plates made from the same solution.

9. RESULTS

① NUMBER OF EXPERIMENT	② INITIAL PHAGE CONCENTRATION PER ML.	③ CONCENTRATION OF INFECTIVE CENTRES PER ML.	④ PERCENTAGE ADSORPTION	⑤ PERCENTAGE RECOVERY	⑥ PERCENTAGE CHANGE FROM CONTROL TO EXPERIMENT	⑦ SIGNIFICANCE LEVEL OF DEVIATIONS BEING DUE TO CHANCE
9b	$3{\cdot}66 \times 10^{7}$	2×10^{7}	54·6%	NOT APPLICABLE	+14·3%	$<0{\cdot}1$
16a	$2{\cdot}23 \times 10^{7}$	$2{\cdot}22 \times 10^{7}$	99·5%	NOT APPLICABLE	−28·0%	$<0{\cdot}001$
18b	$2{\cdot}07 \times 10^{7}$	$2{\cdot}70 \times 10^{7}$	130·4%	NOT APPLICABLE	−13·5%	$<0{\cdot}1$
20b	$9{\cdot}16 \times 10^{6}$	$8{\cdot}1 \times 10^{6}$	88·4%	NOT APPLICABLE	+34·3%	$<0{\cdot}05$
21a	5×10^{6}	$4{\cdot}79 \times 10^{6}$	95·8%	NOT APPLICABLE	−95·7%	$<0{\cdot}001$
22a	$6{\cdot}25 \times 10^{6}$	$4{\cdot}75 \times 10^{6}$	76·0%	141·6%	+6·5%	$<0{\cdot}4$
22b	$6{\cdot}25 \times 10^{6}$	$7{\cdot}34 \times 10^{6}$	117·4%		+55·0%	$<0{\cdot}05$
24a	$5{\cdot}63 \times 10^{6}$	$5{\cdot}18 \times 10^{6}$	92·0%	147·7%	+5·0%	$<0{\cdot}002$
24b	$5{\cdot}63 \times 10^{6}$	$7{\cdot}90 \times 10^{6}$	140·3%		−8·3%	$<0{\cdot}4$
25a	7×10^{6}	$6{\cdot}70 \times 10^{6}$	95·7%	NOT APPLICABLE	+ 3·5%	$<0{\cdot}01$

TABLE 7. ABBREVIATED TABLE OF RESULTS OF EXPERIMENTS IN ANTENNA AND WAVEGUIDE FIELDS.

NOTES:

(1) Selected experiments drawn from the complete table of results in Appendix 8 are presented here. The basis of selection of experiments was as follows:
(a) Only those experiments in which the % adsorption lay between 50% and 135% were selected. Adsorptions of over 100% are of course impossible, however, in view of the degree of accuracy of measurement, these experiments were felt to be satisfactory.
(b) Those experiments (10 to 15) which were performed when the titre was low due to contamination were suspect and were thus rejected.
Data as to t values, percentage changes etc., can be found in the complete tables in Appendix 8.

(2) The initial phage concentration is the concentration of the phage in the test tube which was irradiated and in which infections occurred. It is obtained by multiplying the initial dilution from the stock by the stock concentration. (See note 6).

(3) The concentration of infective centres is the concentration of

infected bacteria in the test tube immediately after the ten minute adsorption and radiation period. It is found by dividing the final plaque count by the dilution after radiation.

(4) The percentage adsorption is the ratio of column 3/column 2 expressed as a percentage. Along with column 5 it is a measure of the validity of the experiment, and shows how many of the phage present did in fact adsorb onto bacteria.

(5) The percentage recovery is a measure of how many of the original phage actually appeared as plaques. This figure can only be calculated in the case of the complementary experiment involving chloroform and antiphage serum. Again, it should not be more than 100% but the arguments in note 1 apply here too.

(6) This column is self-explanatory apart from the fact that a +ve sign means an increase in infections and a -ve sign means a decrease.

(7) The significance level is discussed in Appendices 7 and 8.

TITRE MEASUREMENTS:

EXPERIMENT NO.	TITRE: CALCULATED OR MEASURED ①	STOCK CONCENTRATION ②	TOTAL DILUTION ③	ADJUSTED TITRE ④
3	MEASURED	2.38×10^{11}	–	–
4	MEASURED	2.86×10^{11}	–	–
6	CALCULATED	2.73×10^{11}	7.50×10^{-11}	20.5
7	MEASURED	1.80×10^{11}	–	–
9a	CALCULATED	1.15×10^{11}	1.00×10^{-9}	115.0
9b,c	CALCULATED	1.15×10^{11}	1.67×10^{-10}	19.2
10	CALCULATED	2.52×10^{10}	2.86×10^{-10}	7.2
11	MEASURED	1.70×10^{10}	2.86×10^{-9}	48.6
12	CALCULATED	1.66×10^{10}	9.09×10^{-9}	151.0
13	CALCULATED	1.65×10^{10}	3.33×10^{-8}	544.0
14	CALCULATED	1.64×10^{10}	2.50×10^{-8}	410.0
15	MEASURED	1.54×10^{10}	2.86×10^{-9}	44.0
16	CALCULATED	8.49×10^{10}	3.33×10^{-9}	282.5
18	MEASURED	1.33×10^{11}	3.33×10^{-9}	444.0
19	MEASURED	1.27×10^{11}	3.33×10^{-9}	423.0
20	MEASURED	2.03×10^{11}	8.33×10^{-10}	169.0
21	MEASURED	1.00×10^{11}	1.00×10^{-9}	100.0
22	MEASURED	5.65×10^{10}	1.25×10^{-9}	70.6
23	MEASURED	1.38×10^{11}	1.25×10^{-9}	172.6
24	MEASURED	1.08×10^{10}	1.25×10^{-9}	13.5
25	MEASURED	3.68×10^{11}	2.00×10^{-9}	736.0

TABLE 8. RESULTS OF TITRE MEASUREMENTS

NOTES:

1) "Calculated or measured". This column distinguishes those values of stock concentration which were measured, from those which were estimated using the graph and linear interpolation.
2) "Stock concentration". This is the apparent value of the concentration of the phage and is in virus/millilitre.
3) "Total dilution". In this column are listed the total amounts of dilution from stock to plating employed in the control and experimental samples of the experiment numbered. This dilution is not necessarily the same as that used in determining the titre.
4) "Adjusted titre". By multiplying the concentration by the dilution, the number of plaques expected assuming 100% adsorption is obtained.

Of course, this figure is very dependent on the actual concentration value used, and is therefore not necessarily correct. However, it does provide some basis of comparison with the values obtained in an experiment. (See also note (11) under table 8).

5) The graph is a plot of the apparent concentration of the phage against time.

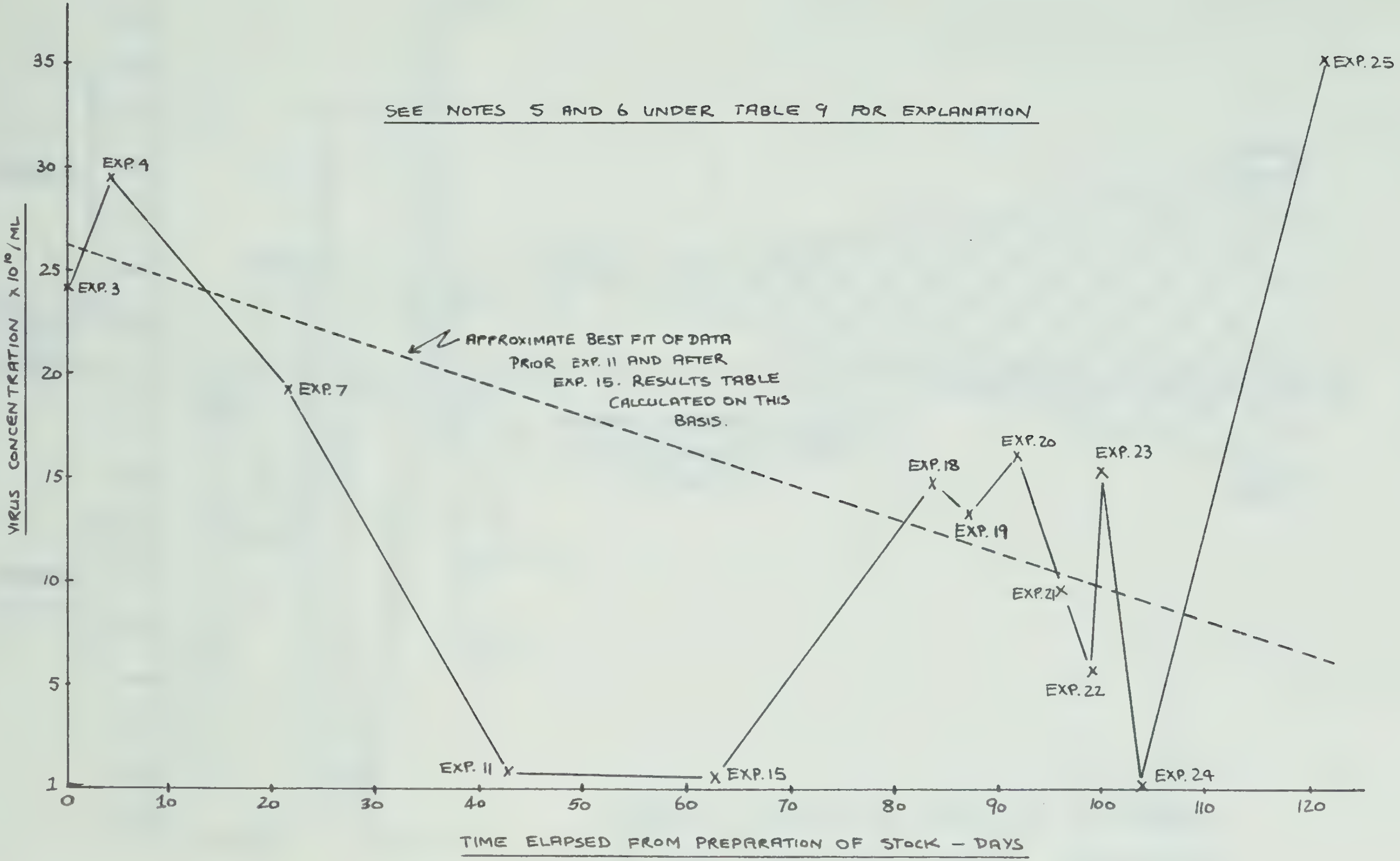
Ideally, the phage concentration should have started at a high value and then slowly decreased with time. One possible explanation for the unexpected (and very troublesome) fluctuations in phage titre is that a clumping phenomenon is at work. If a number of phage clumped together, one would expect an apparent drop in titre, which could perhaps be counteracted by shaking, and this was in fact tried from experiment 15 on, however, the wide fluctuations still persisted.

It should be pointed out here that although the graph suggests more rapid fluctuations in later experiments, this is not necessarily the case as there are more measured points in this latter section.

This characteristic of varying titre seriously hampered efforts to obtain a more complete picture of the behaviour of the system at varying phage concentrations at the time of radiation, and it will be necessary to solve this problem if a more careful analysis of the system is to be made.

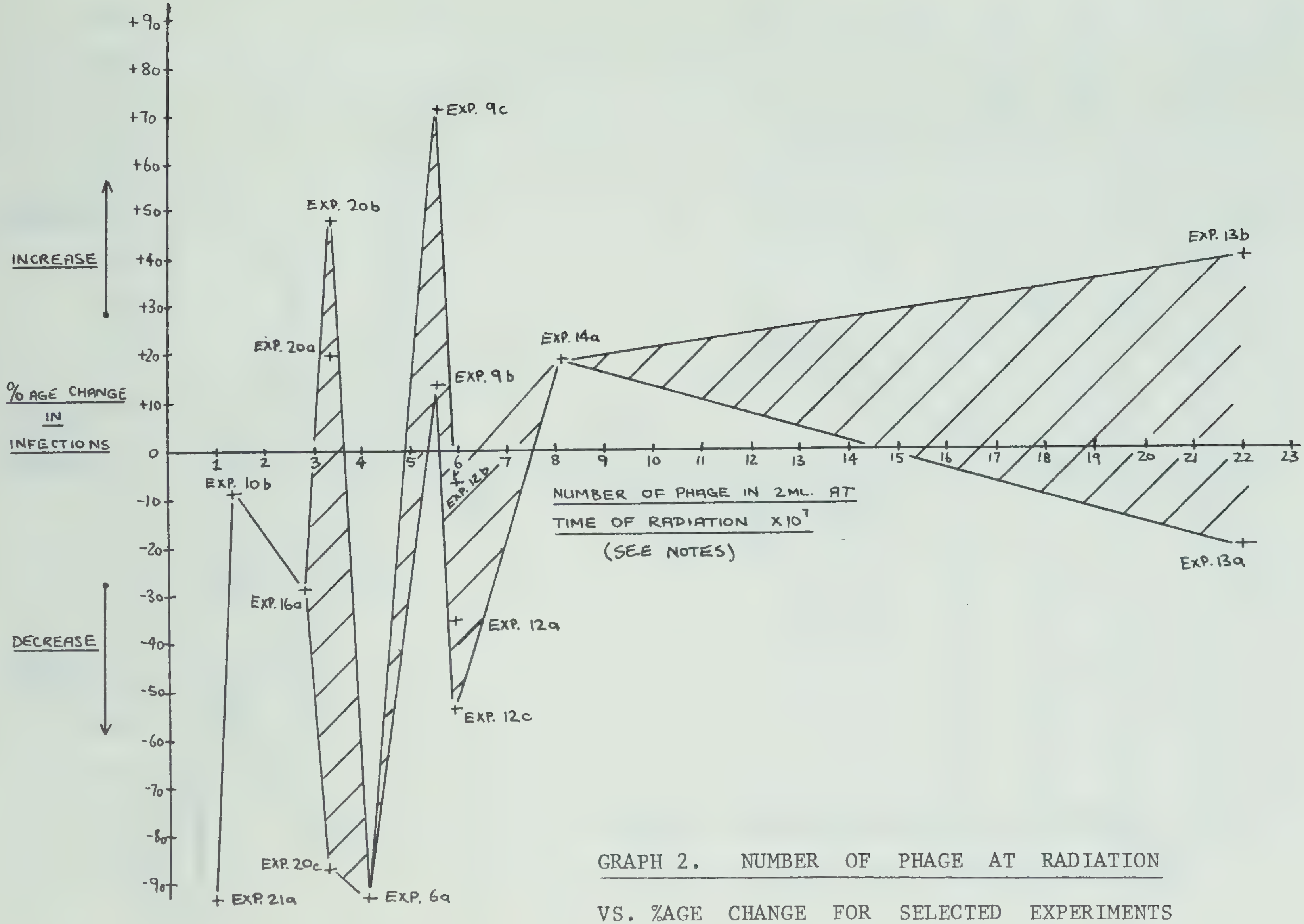
6) The increase after experiment 15 has two possible causes.
(a) At this point, some contamination of the phage stock was noted, and this was removed by chloroform treatment and centrifuging.
(b) The protocol was modified to include a period of shaking and warming of the phage stock before use.

GRAPH 1. CHANGE OF CONCENTRATION OF PHAGE STOCK WITH TIME.

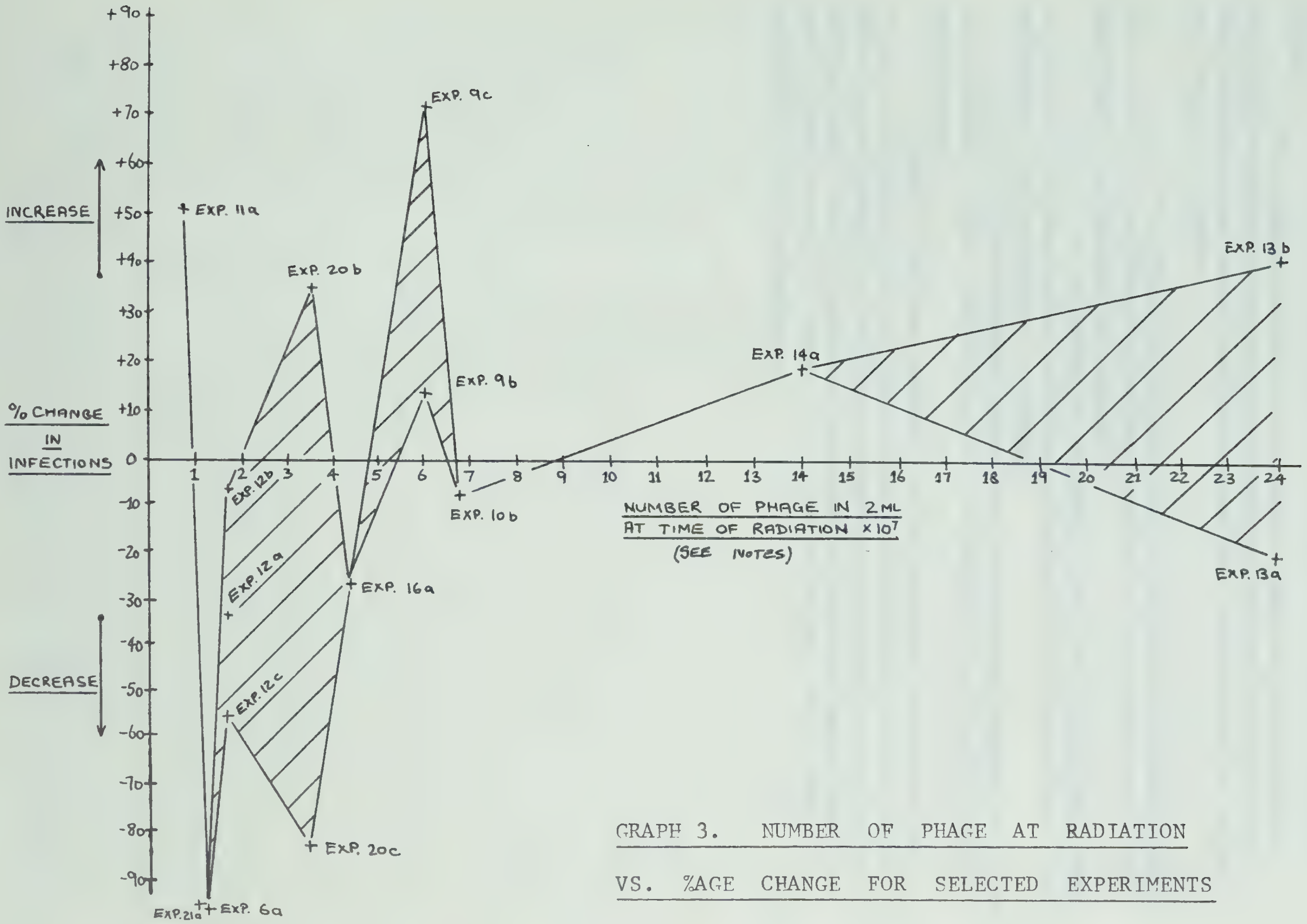


SEE NOTES 5 AND 6 UNDER TABLE 9 FOR EXPLANATION

APPROXIMATE BEST FIT OF DATA PRIOR EXP. 11 AND AFTER EXP. 15. RESULTS TABLE CALCULATED ON THIS BASIS.



GRAPH 2. NUMBER OF PHAGE AT RADIATION VS. %AGE CHANGE FOR SELECTED EXPERIMENTS



GRAPH 3. NUMBER OF PHAGE AT RADIATION VS. %AGE CHANGE FOR SELECTED EXPERIMENTS

NOTES:

The graphs indicate percentage change in the infection rate for different phage concentrations at the time of radiation. Only certain experiments have been selected, the criteria of selection being as follows:

(i) If a measured and adjusted titre value is approximately the same or higher than the values obtained in the experiment, the experiment was considered acceptable

(ii) If an approximate adjusted titre (ie. calculated) was at least of the same order as values obtained in the experiment, the experiment was considered acceptable.

(iii) If the adjusted titre was considerably higher than the experimental values, indicating very low adsorption the experiment was rejected.

Graph 2 has been plotted on the basis of concentration values calculated from the graph of concentration vs. time. The other graph (graph 3), utilizes concentration values calculated by working back from the number of plaques in the experiment, (see note 2 under table 7.) These two graphs represent the probable extremes of this parameter.

10. POSSIBLE IMPROVEMENTS IN APPARATUS AND TECHNIQUE

TECHNIQUE

As previously mentioned, one of the first improvements that could be made to this experiment would be to collect a greater diversification of data about enviromental conditions at the time of the experiment. Some of the variables which may have an effect on the experimental results include time of day, temperature, humidity, magnetic storms, age of the phage (the same stock was used for a duration of six months), composition of growth media, earth magnetic fields. lighting (for instance, this varied from shielded room to waveguide), and stray fields produced by unrelated apparatus in the vicinity of the experiment. These and other possible variables are mentioned in (1).

It appears evident from the results that the concentration of the phage at the time of radiation is a parameter of great importance. Only over a small range of this concentration did the experiment appear to produce inhibitions. During the course of the experiments, great difficulty was experienced in achieving this concentration on different occasions, and this could perhaps have been overcome by using several separate runs at different concentrations on each occasion. The concentration of the E, coli B also appeared to be of some importance, and that of the K12(λ), when used, was definitely important. The use of an optical density meter to check the concentrations of these cultures would have been advantageous. Records of these parameters would permit control of yet another variable.

Although in each case the period of radiation was nominally

ten minutes, this varied somewhat owing to the difficulties in performing the many operations required. The variation was never more than 30 seconds but a record of these variations may have proved useful. One further improvement that could be made to the biological side of the experiments would have been the inclusion of a systematic means of shaking samples whenever they were mixed, therby ensuring homogeneity.

APPARATUS

Even in its final form, the apparatus employed here is rather limited. One of the first improvements would be to provide a more powerful and flexible source of microwave power. The particular instrument used here was only capable of approximately 5 watts output up to a frequency of approximately 2.5 Ghz. It did not operate satisfactorily at power levels below 1.5 watts, and there was no provision for a feedback loop to level the power output. Its pulsing characteristics were poor, and consequently continuous re-adjustment of the controls was necessary in order to maintain the desired waveshape. Tuning the generator to a particular frequency was a rather complicated procedure, and performing experiments at different frequencies, even in a small band, would have been extremely difficult. One other disadvantage of this particular generator was its sensitivity to line voltage changes, both in power and frequency of output.

A full program of experiments on the phage - *E. coli* system should include checking the effect of varying frequency, say in the range from 300 Mhz. to 10 Ghz., and perhaps even higher. Several arguments exist for this choice of frequency band, and further, for

the choice of particular frequencies within this band. For instance, radiation from the galaxy is very high at frequencies below 300 Mhz., and living organisms could be expected to have adapted to background radiation at these frequencies. In the region of 2 Ghz., however, there is almost no galactic radiation, and low powers could thus have considerable effect in this frequency range. Using similar arguments, an upper frequency limit can be set by the earth's atmospheric radiation which is constant from about 300 Mhz. to 10 Ghz., above which it rises sharply. Thus, this "galactic window" in the region of 2 Ghz. is apparently the most interesting frequency band, (13).

Power levels should be variable at least from 0.1 watts to 50 watts, especially if experiments in the radiation field are contemplated. Pulse modulation from 0 to 100% duty cycle should be possible and the pulse repetition rate should also be variable from a few Hz. to, say, 100 Khz. Particular carrier and pulse-repetition frequencies should be easy to establish.

Above all, the generator (or generators, as several would be required to cover the above frequency range) should have levelled power output, and should be insensitive to line voltage variations.

A single antenna system would be impossible over a large frequency range, and waveguide radiation would only be feasible above about 1 Ghz. In the present experiment, average power density levels were in the region of 1 to 10 mW/cm^2, however, it is essential to perform the experiment with the average power density in the 10 μW/cm^2 region and at various values within the range between 100 μW/cm^2 and 10 mW/cm^2. Power densities greater than 10 mW/cm^2 would also be of

interest but great care would have to be taken to prevent heating, perhaps by using larger peak powers and shorter duty cycles.

For the above reasons, it is necessary to perform radiation experiments in certain ranges in the radiation field of an antenna. The above quoted maximum generator power of 50 watts would only be useful in the case of near-field radiation, and an improvement, if not in results, then at least in power density measurement, could be obtained with far field radiation. Appendix 2 covers the relevant power output calculations, the power required being of the order of 0.5 KW. With this power level, great care would have to be taken to prevent injury to personnel, and to minimize reflections so that a known power density could be obtained. To this end an anechoic chamber would have to be designed, and such a design is presented below. By putting the anechoic chamber into a controllable environment, for instance a plant-growth chamber, it would be possible to maintain a specific temperature, humidity and type of lighting and the effect of these variables could then be studied.

One final improvement that could be made to the apparatus is the provision of a means of simultaneous power measurement, both before and after the sample holder in the case of the waveguide radiation system. This would enable the power actually absorbed by the sample to be easily measured, thereby giving an indication of the possibility of temperature rise.

DESIGN OF AN ANECHOIC CHAMBER

If experiments in the far-field of a microwave antenna are

contemplated, high power levels are required and so some means of containing the radiation is necessary, especially from the point of view of the safety of the personnel involved. Further, in order to improve the accuracy of measurement of the field established, it is essential that reflections be minimized, and hence an anechoic chamber is indicated. Such a design is presented in Appendix 6, reflections being sufficiently low so that the following measurement accuracies can be guaranteed:

1 Ghz. ± 1 dB, 3 Ghz. ± 0.3 dB and 10 Ghz. ± 0.2 dB.

The specifications of a slightly improved version of the chamber are also presented, measurement accuracy in this case being guaranteed to:

1 Ghz. ± 0.5 dB, 3 Ghz. ± 0.2 dB and 10 Ghz. ± 0.1 dB.

The design and specification refer to communications with the firm of Emerson and Cuming Inc., Microwave Products Division, Canton, Massachusetts.

11. LINES OF FURTHER POSSIBLE RESEARCH

PHAGE-E.COLI SYSTEM

As previously mentioned, there are several avenues of further research into the effect of microwave radiation on the phage a42 - E.coli B system.

(1) The effect of varying the phage concentration at the time of radiation should be carefully checked by deliberately repeating the experiment with different concentrations.

(2) Measurement of the initial bacterial titre, either by means of an optical density meter or by use of titration methods should be made.

(3) The pulse duty cycle, pulse repetition rate and peak and average power densities should be varied through wide limits in order to check the effect of these parameters. (See discussion).

(4) The carrier frequency of the microwave radiation may or may not be important, and so this should be varied over a wide range to see whether the effect is enhanced at any particular frequency. In the discussion below, the possibility of the existence of resonance phenomena suggests some frequency dependence of the effect.

Having examined the effect of these parameters on the phage a42 - E.coli B system, it would be interesting to choose different strains of phage and E.coli and see if the change in genotype has any quantitative or qualitative effect on infection rate. It is quite likely that some genotypes will be more sensitive to

radiation than others, and it should be possible to select for the most sensitive genotype. Other virus - bacteria systems exist, and these could also be radiated to see if any effect was obtained.

MAMMALIAN CELLS, SKIN GRAFTS, ETC.

The discussion below suggests that perhaps a bond-breaking phenomenon in surface-to-surface reactions is responsible for the effect observed. By choosing other systems with such surface-to-surface reactions, and irradiating them, this hypothesis could be checked.

Mammalian skin-grafts involve surface-to-surface reactions and the effect of microwave radiation on such a skin-graft would be very interesting indeed. The possibility exists that either the skin-graft will be completely rejected, or rejection of the skin-graft may be prevented, since the rejection phenomenon involves a surface-to-surface reaction. If this latter case were true, microwave irradiation of the type described here would have possible application in the field of organ rejection and immunology.

NEMATODES

Another purely biological system which was suggested for radiation is that of the development of simple organisms such as free living nematodes (worms). These creatures comprise a relatively small number of cells (500±50) and any anomalies in the full grown worm can be traced back to a particular cell division. Thus, if the radiation had any effect on development, it would be possible to determine where, and perhaps why, this effect ocurred. Since

nematodes are small, a number can easily be placed in a waveguide and radiated for a period of several days.

As an interesting sideline to the phage-E.Coli investigations, such an experiment was actually performed using the nematode, Panagrellus redivivus L.

A piece of waveguide into which depression slides could be inserted, and subsequently manipulated, was connected to the same microwave generation apparatus as before, with the exception that the electric field was arranged horizontally instead of vertically. Since the water in the depressions was very shallow, this change ensured maximum coupling to the samples. A microscope and closed-circuit television camera were arranged so that direct observation of the surface of the slide was possible. Nematodes, singly, or in small numbers, were now introduced into the slide depressions, and observed on the television screen.

At this stage, the microwave source was switched on, and the various parameters were adjusted to determine which produced maximum effect. In general the rhythmic sinusoidal motion of the worms was upset, and under certain conditions of radiation, the nematodes appeared to align themselves perpendicular to the electric field in an hitherto unobserved type of motion. The important radiation parameters were found to be pulse length and pulse frequency.

At the present time, there is no known recepter organ on the worm, however, apart from the aligning phenomenon, effects are typical of worms whose nervous systems have been damaged by finely focused laser beams (6). Of great importance, however,

is the fact that the nematodes usually return gradually to normal on cessation of the radiation.

An interesting adjunct to locomotion studies would be an investigation into the fecundity of nematodes in the presence of microwave radiation. Studies of possible induced genetic changes should have fruitful results in this connection, and undoubtedly many other interesting experiments could be devised using the nematode.

ENZYMES

All the above experiments have one disadvantage in common and that is that they take at least 24 hours, and more probably several days before results can be obtained. A reaction which is dependent on surface-to-surface effects, but which is completed in ten minutes or so has been proposed. This system comprises a reaction employing a horse-radish peroxidase compound (enzyme), and is described in (4). Basically, the reaction is as follows:

Using a ferrocyanide substrate, hydrogen peroxide is added to the horse-radish peroxidase. As the reaction proceeds, the ferrocyanide is converted to ferricyanide which has a completely different absorbtion spectrum from ferrocyanide. An optical density meter can thus be used to follow the course of the reaction. The time to completion is dependent on the strengths of the chemicals employed, and in this case would be of the order of 10 minutes. Two samples would be employed, one as a control and one as a test. The test sample would be radiated while the reaction was proceeding,

and any change in the amount of ferrocyanide produced would be a measure of the effectiveness of the microwaves. Results could be obtained in a matter of 15 minutes or so.

ELECTROPHORESIS OF PROTEINS

A final system which has been suggested is the electrophoresis of proteins. Changes between an irradiated electrophoretic apparatus, and one used as a control, would constitute a measure of the effectiveness of microwave radiation in disturbing this reaction.

Many other reactions involving surface-to-surface effects can doubtless be found, and the effect of microwave radiation on these may prove most interesting..

12. DISCUSSION

A careful study of the experimental results and graphs presented in chapter 7 reveal a rather striking dependence of change in infection rate on number of phage present at radiation. Of course, the dependence of the form of the graphs on the actual values chosen necessitates caution in drawing conclusions from them. Since the values actually chosen probably represent limits of the number of phage at radiation in each case, the true picture probably lies somewhere in between.

Bearing the above in mind and referring to graph no. 2., it seems that the range of values between 1 x 10^7 and 4 x 10^7 produces the most consistent reduction in number of infections, though there are some cases in which an increase is obtained in this range. Changes of over 90% are common and this suggests that some effect is definitely present.

A possible explanation for the upper limit is that the kinetics of the phage - E. coli reactions become uncertain when there are more than one phage per 10 bacteria. Multiple infection (2 or more phage infecting one bacteria) often results and the final plaque count can be far from accurate. In addition Prof. McWhirter suggests that though these kinetics have been adequately worked out with regard to the system in a biological context, some modification may be needed in the type of experiment considered here.

The number of 4 x 10^7 represents approximately one tenth the number of bacteria present in 2 ml of a good overnight culture

(concentrations of 2×10^8/ml) and so the effect of using a poor overnight culture would be similar to that of having too high a concentration of phage. This may explain the failure of some of the experiments in which the phage concentration was in the above range. Titration of the bacteria or optical density measurement should have been performed as a check on this parameter.

There is insufficient evidence to draw definite conclusions as to the lower limit of the range and it may well be that these results are also due to some other factor such as low bacteria concentration. If indeed a lower limit does exist then no apparent explanation as to why is as yet forthcoming.

Quite a number of experiments appeared to produce an increase in the number of infections, although in most cases, the increase only appeared when the phage concentration was above 4×10^7/ml or below 1×10^7/ml. Outside this range, increases and decreases seem to occur randomly. Again, there appears to be no explanation for this as yet.

On the assumption that the above anomalous effects were due to some unconsidered variable, an attempt was made to produce an hypothesis to explain the reduction of infections.

The mechanism of the phage-bacteria infection was described in a previous section and is briefly repeated here. The long protein tail fibre of the phage adsorbs onto the surface of a bacterium, and the phage's hereditary material is injected into the bacterium where it grows until the bacterium bursts. Now, since ne ther phage

nor bacteria appear to be adversely affected by the microwave radiation, as concluded in chapter 8, some other inhibition mechanism must be at work. It seems that the most likely mechanism is some sort of resonance effect, especially considering the low energy levels involved. Could it be that the protein tail fibres of the phage are made to vibrate, thus making it difficult for the necessary adsorbtion to take place ? If this were the case, some frequency dependence would be expected though it would probably be fairly broad - band owing to the low Q of the system due to damping by the surrounding medium and the physical nature of the phage tail. It may be that the whole bacterium or phage is being made to vibrate, with similar effect. A calculation of resonant frequency based on the size, shape and mass of these components may shed some light on these possibilities, though such a calculation would be difficult to perform with presently available data.

The result of experiment 6 suggests a definite dependence on pulse length, ie. the longer the pulse length the greater the effect. Of course, this may be due to the average power being higher, but it is also possible that, while the pulse is "on", infection is inhibited, but during the "off" time, infections can take place. Presumably, infection time has some time constant depending on phage and bacteria concentration, and thus, the longer the "off" time, the more infections that can take place. It seems that once an infection has taken place, the radiation has no effect on the further course of events.

Results with continuous radiation were unsatisfactory in that they produced little change in infections, although data are insufficient to establish this conclusively. Thus it seems that the sudden change from a condition of no radiation to one with radiation is also important in reducing the number of infections.

As can be seen in tables 7 and 8, several cases exist in which the titre value is far lower than the plaque counts for the control. Ideally, the control plaque count should be some 60 to 70% of the titre value if the experiment is to be convincing. Although several of the experiments which were plotted on the graphs, and have been used in drawing conclusions, did not satisfy this criterion, their use is justified by the degree of uncertainty of the titre value.

Initially it was thought that the incompatible titre and control plaque counts were a result of premature bursting in the control samples, and, as described under the "K12(λ) Scavenging" section of chapter 7, steps were taken to reduce this possibility. A cure was not effected, however, and so the possibility of coagulation of the virus arose. Shaking the virus before use, and revised dilution procedures proved ineffective and so the problem still remained.

In an attempt to account for both the adsorbed and unadsorbed phage and reduce this problem, the protocol was further modified as described in chapter 7 and in note 10 under table 8. Again, incompatible results were obtained, the plaque counts in the case of scavenging with anti-phage serum being far too high.

Consider experiments 22 to 24. In no case do the control plaque counts in "a" and "b" add to give the titre value, as they should. One would expect the control plaque count in the case of scavenging with $CHCl_3$ to be zero, or at least very small relative to the titre, as is the case in experiments 21 and 25. Thus, conclusions are difficult to draw here.

Experiment 21, however, suggests a large decrease in infections under the action of radiation, as would be expected from what has gone before. (The number of phage at the time of radiation is just inside the 1 to 4 x 10^7 range). Experiment 25 suggests a very slight increase in the number of infections, again as would be expected with the number of phage (7 x 10^7) outside the postulated range.

At this stage, there is no explanation as to why titre and control plaque counts should be incompatible.

CONCLUSIONS:

The most obvious conclusion from the experimental results is that, in general, microwave radiation modifies the rate of infection of the bacteria, E.coli B,by the phage, a-42.

Under certain conditions of concentration, the effect usually appears to be inhibiting, however, increases in infection rate are almost equally common over the range as a whole. Results with higher relative phage concentrations are apparently less significant for kinetic reasons, and so there is some justification for claiming that the effect is inhibitory.

The experiment reveals several hitherto unsuspected variables, and it is likely that there are others. This suggests that all possible parameters should be carefully recorded and held invariant if possible.

One of the major problems encountered in the experiment was that of obtaining a desired phage concentration, and any further investigations will require that this be achieved. With this problem solved, experiments using anti-phage serum and chloroform simultaneously, as was done in the later experiments, should provide very convincing results.

The experiment establishes some of the desirable characteristics of an environmental chamber for use with microwave radiation. Among these are an anechoic chamber if far field experiments are contemplated; high power microwave generating equipment with precise

frequency and power level control, and variable pulsing characteristics, is also required. An ability to maintain temperature, humidity and other environmental factors would be advantageous.

Experiments using waveguides to radiate samples impose less stringent conditions on the generating equipment from the point of view of power output. Of great importance, however, is a carefully designed and constructed arrangement in which to mount the samples in the waveguide, the key problem being in the measurement of the power density in the region of the sample.

Further development of precise means of measuring small rises in temperature would also be fruitful. It seems that the electronic thermometer previously described is a good starting point, though a design employing a thermocouple would likely be very good too. A careful evaluation of the two principles involved is indicated.

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APPENDIX 1

VARIATIONS IN THE NEAR FIELD OF AN ANTENNA

In view of the relatively high power required for far field operation, see Appendix 2, the antenna field experiments were carried out in the near field. Some of the difficulties involved in measuring the near field power density have been mentioned elsewhere, perturbations due to the sample and measuring device being the most important factors. Not previously mentioned, but also of great importance, is the rapid variation of power density in the near field region. Fig. 9 gives some indication of this variation (60).

The particular positions of minima and maxima are dependent on the amount perturbations of the field, and so it may be that a measuring instrument causes a maximum while the sample causes a minimum in the same place. The measured power density would then be incorrect.

Although the power density is elsewhere stated as being 6.5 mW/cm^2, this value could be considerably in error and it is therefore only an order of magnitude. Frey (15) has made similar statements regarding some of his measurements, indicating that this type of uncertainty has to be tolerated in antenna field measurements.

The waveguide radiation experiments were commenced in an attempt to solve this problem which is discussed at length in (1).

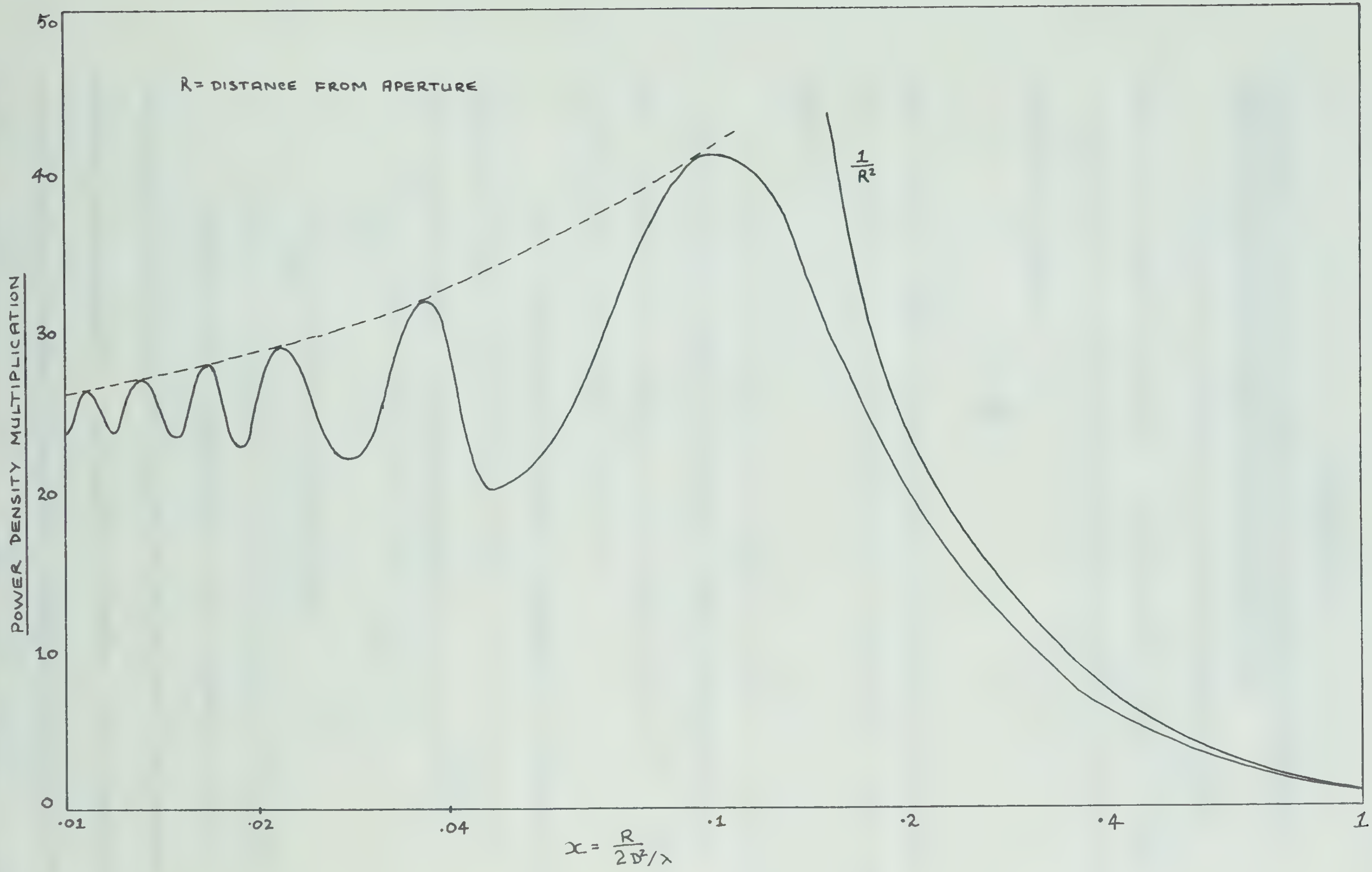


FIG. 9 AXIAL POWER DENSITY IN THE NEAR FIELD NORMALIZED TO UNITY AT $2D^2/\lambda$.

APPENDIX 2

CALCULATIONS OF THE POWER DENSITY IN THE FAR FIELD AND THE POWER LOAD REQUIRED TO ESTABLISH THIS FIELD (3)

It is first necessary to ascertain where the far field of an antenna commences. The generally accepted formula for calculating at what distance the Frauhenhofer or far field conditions begin is:

$$R \geqslant \frac{2d^2}{\lambda}$$

where d is the largest characteristic dimension of the antenna (the aperture diagonal in the case of a horn) and λ is the wavelength.

If the application of an anechoic chamber is envisaged, or indeed, if any kind of reasonably small screened is to be used, a limitation is placed on the maximum value of R. Thus d is limited for a particular value of λ.

Now, in the anechoic chamber presented in Appendix 6, the maximum value of R is about 10 feet. At a wavelength of 12.25 cms (2450 Mhz), the maximum value of d is thus:

$$d^2_{max} = 12.25 \times \frac{10}{2} \times 12 \times 2.54 = 1860$$

$$\therefore d_{max} = \underline{43.0 \text{ cms. Say } 40 \text{ cms.}}$$

Since d is limited, so also is the gain of the horn.

Consider now a symmetrical horn as shown in Fig. 10. The length of the taper is limited by the size of the antenna mounting arrangements of the anechoic chamber. This applies also to the aperture; however, there is no problem with the latter as the far field limitation of d is rather more stringent. Assume

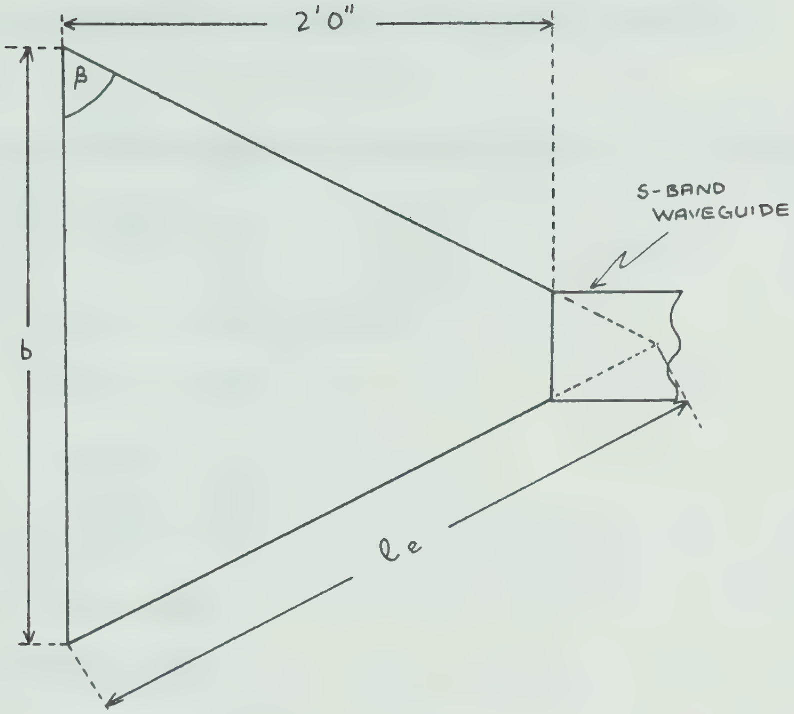


FIG. 10a WAVEGUIDE HORN - TOP ELEVATION

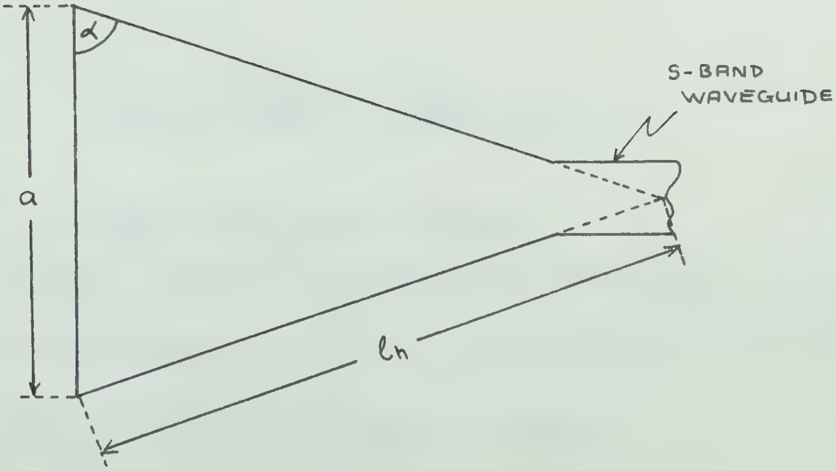


FIG. 10b WAVEGUIDE HORN - SIDE ELEVATION

also that a and b are in the same ratio as the waveguide dimensions.

Now, S-band waveguide dimensions are 7.2 x 3.4 cms.

$$\therefore \frac{b}{a} = \frac{7.2}{3.4} = 2.12$$

$$\text{Also } d^2 = a^2 + b^2 = a^2 + (2.12a)^2$$

$$= a^2(1 + 4.48)$$

$$= 5.48\ a^2$$

$$\text{Hence, } a = \frac{1600}{5.48} = 292$$

$$\text{i.e. } a = \underline{17.1 \text{ cms.}}$$

$$b = \underline{36.3 \text{ cms.}}$$

Now,

$$\tan \alpha = \frac{2 \times 12 \times 2.54 \times 2}{(17.1 - 3.4)} = \frac{121.8}{13.7}$$

$$= \underline{8.88}$$

$$\text{Thus, } \ell_h = \frac{a}{2\cos\alpha}$$

$$= \frac{17.1}{2 \times 0.113} = \underline{75.6 \text{ cms.}}$$

Also,

$$\tan\beta = \frac{2 \times 12 \times 2.54 \times 2}{(36.3 - 7.2)} = \frac{121.8}{29.1} = \underline{4.16}$$

And so,

$$\ell_e = \frac{b}{2\cos\beta} = \frac{36.3}{2 \times 0.233} = \underline{78.0 \text{ cms.}}$$

Now, the gain of a horn antenna is given by:

$$G = 10\ (1.008 + \log \frac{a}{\lambda} \cdot \frac{b}{\lambda}) - (L_e + L_h) \text{ dB}$$

Where L_e and L_h are obtained graphically from:

$$s = \frac{b}{8\lambda l_e} \text{ and } t = \frac{a}{8\lambda l_h} \quad (3)$$

Hence, $s = \frac{36.3^2}{8 \times 12.25 \times 78}$ and $t = \frac{17.1^2}{8 \times 12.25 \times 75.6}$

$= \underline{0.172}$ $\qquad = \underline{0.039}$

From the graphs (3), we get $L_e = 0.8$ dB, $L_h = 0.2$ dB.

Hence, $G = 10\ (1.008 + \log \frac{17.1 \times 36.3}{(12.25)^2}) - 1$ dB.

$= (10.08 + 10 \log 4.14) - 1$ dB.

$= 10.08 + 0.617 \times 10 - 1$ dB.

$= \underline{15.25 \text{ dB. (or 33.5 times)}}$

Having calculated the maximum antenna gain available under the circumstances, the power input required to produce a power density of 10 mW/cm^2 at the rear wall is now found as follows:

$$P = \frac{W_t . G_t}{4\pi R^2} \times 1000$$

Where P = power density, mW/cm^2

R = distance in direction of maximum gain, cm.

G_t = antenna gain

W_t = power input, watts.

$$\therefore W_t = \frac{4\pi R^2 P}{1000 G_t} = \frac{4\pi \times (120 \times 2.54)^2 \times 10}{1000 \times 33.5}$$

$= \underline{349 \text{ watts.}}$

If use of power densities of up to 50 mW/cm^2 are contemplated, a power input of 350 x 5 = <u>1750 watts</u> is called for, though this is not very practical.

APPENDIX 3

POWER DISTRIBUTION IN THE WAVEGUIDE

In the system in use in this experiment, the rectangular S-band waveguide is excited in the TE_{10} mode. This gives rise to a field distribution like that shown in Fig. 11. The distribution is sinusoidal.

Now, since power is proportional to E^2, the total power across the guide is given by:

$$P_{tot} = A\int_{o}^{b} E^2\,dx, \text{ where A is the admittance.}$$

Further, $E = E_o \sin \frac{\pi x}{b}$ where x is in the direction of b

$$\therefore P_{tot} = A\int_{o}^{b} E_o \sin^2 \frac{\pi x}{b}\,dx$$

$$= \left[\frac{x}{2} - \frac{b \sin(\frac{2\pi}{b})x}{4\pi}\right]_{o}^{b} E_o \cdot A$$

$$\underline{= A\frac{b}{2}\;E_o}$$

Let a test tube now be inserted into the guide in the manner shown in Fig. 12. The power impinging on this test tube is given by:

$$P_{tube} = A\int_{\frac{b}{2}-r}^{\frac{b}{2}+r} E_o \sin^2\frac{\pi x}{b}\,dx$$

$$= A\left[\frac{x}{2} - \frac{b \sin(\frac{2\pi}{b})x}{4\pi}\right]_{\frac{b}{2}-r}^{\frac{b}{2}+r} E_o$$

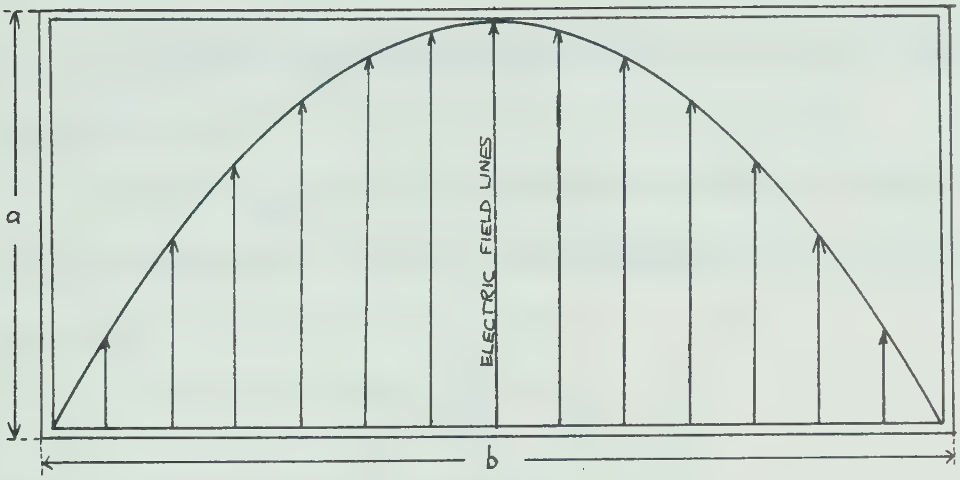


FIG. 11 DISTRIBUTION OF ELECTRIC FIELD IN WAVEGUIDE

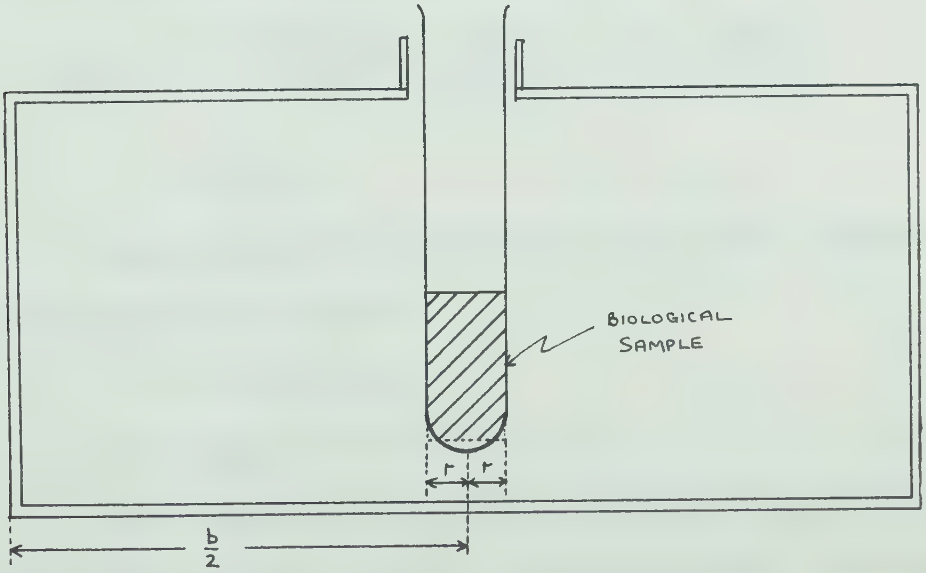
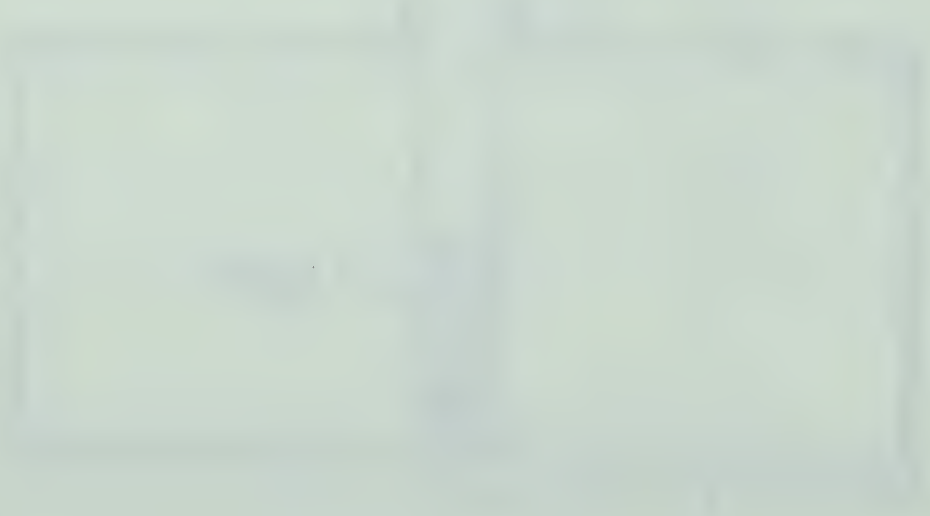


FIG. 12 TEST TUBE INSERTED INTO WAVEGUIDE

[illegible]



[illegible]

$$= A\left\{\frac{b}{4} + \frac{r}{2} - \frac{b}{4} + \frac{r}{2} - \frac{b}{4\pi}\left[\sin(\pi + \frac{2\pi r}{b}) - \sin(\pi - \frac{2\pi r}{b})\right]\right\} E_o$$

$$= A\left[r - \frac{b}{4\pi}\ \sin(\pi + \frac{2\pi r}{b}) - \sin(\pi - \frac{2\pi r}{b})\right]E_o$$

NB This analysis does not allow for the fact that the test tube perturbs the field.

Since P_{tot} is the power value measured on the power meter, it is now possible to find the power impinging on the test tube, as follows:

$$\text{Power to tube} = \frac{P_{tube}}{P_{tot}} \times P_g$$

Where P_g is the power in the guide.

$$\therefore \text{Power} = \frac{r - b/4\pi\left[\sin(\pi + \frac{2\pi r}{b}) - \sin(\pi - \frac{2\pi r}{b})\right]}{b/2} P_g$$

Now, $b = 7.2$ cms., $r = 0.5$ cms., $P_g = 160$ mW.

$$\therefore \text{Power} = \frac{0.5 - \frac{7.2}{4\pi}\left[\sin(\pi + \frac{2\pi}{7.2}) - \sin(\pi - \frac{2\pi}{7.2})\right]}{3.6} P_g$$

$$= \underline{0.263\ P_g}$$

Substituting the value for P_g given above, the total power impinging the tube is:

$$P = 0.263 \times 160 \text{ mW}$$

$$= \underline{42 \text{ mW}}$$

This assumes that the tube and liquid occupy the whole depth of the waveguide, which is not strictly true. Allowing for this factor, the power impinging on the tube is reduced as follows:

Height of liquid = 3.0 cms.

Height of waveguide = 3.4 cms.

$$\therefore \text{Power to tube} = \frac{3}{3.4} \times 42 = \underline{37.1 \text{ mW}}.$$

Not all this power is absorbed in the liquid, owing to the presence of the glass test tube and to reflections from the non-uniformity. However, it is now possible to calculate the power density in the region of the sample.

The power impinging on the 1 x 3.4 cm. strip was found to be 37.1 mW.

$$\therefore \text{Power density} = \frac{37.1}{3.4} = \underline{10.9 \text{ mW/cm}^2}.$$

In the experiments performed, the value of P_g was decided upon as follows:

Power density in antenna field = 6.5 mW/cm^2.

Cross area of waveguide = 7.2 x 3.4 = 24.5 cms^2.

$\therefore$ Total power in guide = 6.5 x 24.5

$\therefore P_g = \underline{160 \text{ mW}}.$

The antenna field power density was used so that the waveguide would be exposed to a power density of the same order as those in the antenna field.

The above calculations show that in practice, the waveguide power density was higher than the power density in the antenna field.

APPENDIX 4

TEMPERATURE RISE CALCULATIONS

In order to calculate the power input to a substance subjected to microwave radiation, the following formula is employed (2):

$$P = \frac{4.2\ m\ c_p . T}{t}$$

Where P = power in watts

m = mass of substance in grams

c_p = specific heat of substance

T = temperature rise, °C

t = time in seconds.

In this particular case, the substance was Luria broth, which is composed very largely of water; c_p can thus be considered to be unity. Since the accuracy of this approximation is not known, the following calculations could be in error in the first decimal place.

Each sample contained 2 ml. of the broth, the cross area of the sample being approximately 3 cm^2. The power density in the region of the sample was approximately 11 mW/cm^2, see Appendix 3, and so the temperature rise can be calculated for a 10-minute period.

$$T = \frac{Pt}{4.2m}\ ^{o}C$$

$$= \frac{3 \times 10 \times 60 \times 11}{4.2 \times 2 \times 1000}\ ^{o}C$$

$$= \underline{2.4\ ^{o}C}$$

This agrees closely with the measured value of 1.4 °C when it is considered that no allowance has been made for cooling, or for the fact that some power is reflected and therefore not absorbed by the broth. Further, the glass is not heated by the microwaves but serves to cool the liquid in it.

APPENDIX 5

THE TRANSISTOR THERMOMETER

INTRODUCTION:

Since temperature changes in biological systems under low power microwave radiation are likely to be small, a thermometer with a resolution of 1/100 °C would be very useful. For this reason, it was decided to design and construct such a thermometer.

THEORETICAL CONSIDERATIONS:

An ordinary silicon transistor has a linear temperature coefficient of 2 mV/°C. In the thermometer application described here, two transistors were employed, one as a constant temperature reference and the other as a measuring element. By connecting the transistors as diodes (see Fig. 13), and passing a current through them, a voltage drop of approximately 0.6V is obtained. This voltage varies with the current passing through the transistors, as well as with their temperatures.

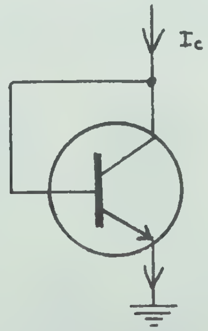


FIG. 13 DIODE CONNECTION OF TRANSISTOR

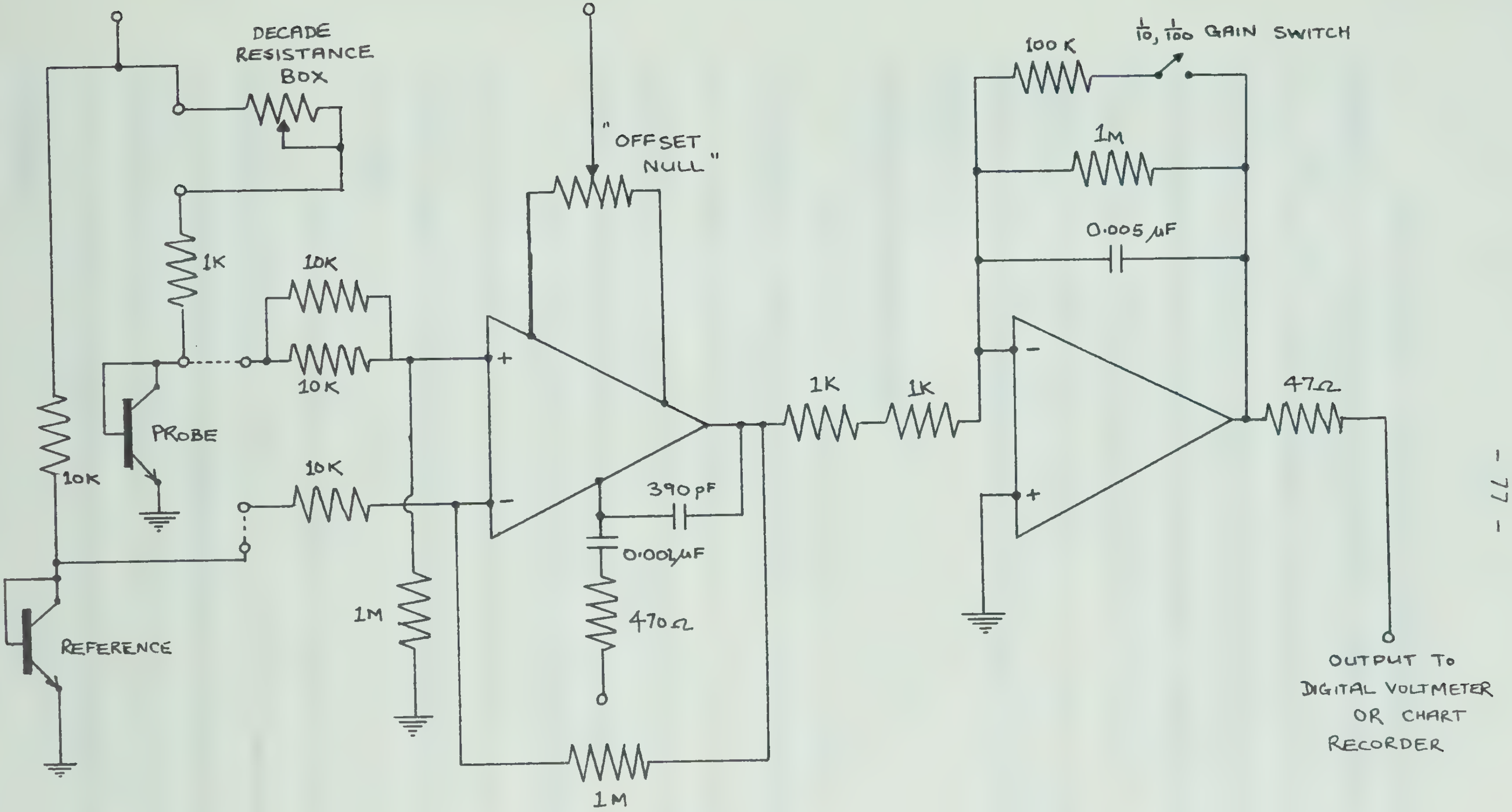


FIG. 14 CIRCUIT OF TRANSISTOR THERMOMETER

Now, in order to measure a temperature change of 1/100 °C, it is necessary to measure a voltage change of $2 \times 10^{-3} \times 10^{-2} = 20\mu V$. Further, the indicating device employed was either a digital voltmeter or a chart recorder, and so it was felt desirable for a 1/100 °C temperature change to give a 1.0V output change. Thus, an amplifier with a maximum D.C. gain of $\frac{1}{20 \times 10^{-6}} = 50{,}000$ was required, and in order to negate the effect of the 0.6V voltage drop, a differential amplifier was indicated.

PRACTICAL CONSIDERATIONS:

Since it will often be necessary to measure changes of the order of 1/10 °C or even 1.0 °C, a range switch would be useful, and such a switch was incorporated. In one position, the amplifier gain is 50,000, and in the other, it is 5,000, this giving a 1V output change for a 1/10 °C temperature change.

Also included are terminals to enable connection of a decade resistance box for adjustment of probe current. In addition, there is a potentiometer to enable the input offset to be nulled.

The circuit finally evolved is shown in Fig. 14. All resistors are 1% metal film types to minimize temperature effects. The operational amplifiers employed were Fairchild μA725B instrumentation types, having high open loop gain, low offset current (2nA), low input voltage drift (0.6μV/°C) and high common mode rejection.

The reference transistor was mounted in a glycol-filled hole in a solid steel cylinder (approximately 6 ins. dia. and 6 ins. high). The steel block was mounted in a styrofoam container which was then placed in a fibreglass-wool-lined garbage can. For all practical purposes, this temperature could be considered insensitive to transient effects.

USE OF INSTRUMENT:

In order to make a measurement, the following procedure was adopted.

(1) The digital voltmeter or chart recorder was connected to the output.

(2) Both inputs were grounded and the output zeroed with "offset null."

(3) The reference transistor, probe transistor and resistance box were connected.

(4) The measuring probe was immersed into the sample and the output zeroed using the resistance box.

(5) The probe was removed and the sample heated as required.

(6) The probe was reinserted into the sample and the change in the voltmeter or chart recorder reading noted.

INSTRUMENT TESTS:

Several tests were performed in order to ascertain the reliability of the instrument. Of prime importance is the stability, and this was determined by putting both probes into the reference block described above, and allowing the instrument to stand for several hours with the chart recorder switched on.

A drift of only a few hundredths of a degree was apparent during this period, the drift over the period of time required for a measurement being negligible.

Fig. 15 indicates the manner in which the gain of the circuit was checked. From the figures shown, the gain is:

$$G = \frac{100 \times 10^3}{10} \times 5 = \underline{50,000}$$

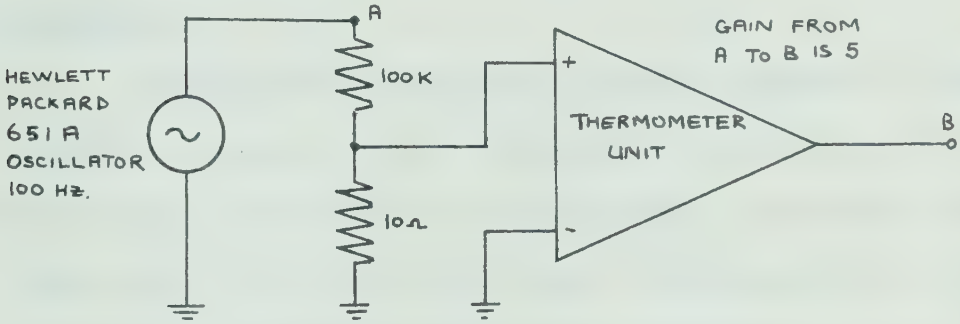


FIG. 15 GAIN TESTS

In order to obtain some indication of the accuracy of the instrument, the following test was performed.

(1) 1 litre of water in a glass beaker was allowed to stand for several hours, thus attaining room temperature. This temperature was measured with a mercury thermometer.

(2) A second beaker of water was heated to a temperature exactly 10°C higher than the first, again using a mercury thermometer for measurement.

(3) The transistor probe was inserted into the 1 litre beaker and a zero established on the chart recorder as described above.

(4) 1 ml. of the heated water was added to the 1 litre beaker.

This should have resulted in a temperature rise of:

$$d\theta = \frac{1}{1000} \times 10 = \frac{1}{100}\,^{o}C$$

(5) 10 ml. of heated water was added to give a 1/10°C rise. In both cases, the temperature rise as measured by the instrument agreed closely with the theoretical temperature rise.

CONCLUSION:

Although more extensive testing is required, it seems a useful instrument has been developed. One drawback of the thermometer in its present form is the probe size, this being approximately 1/8 in. dia. and 6 ins. long. For measurements on very small samples, a smaller probe will be necessary.

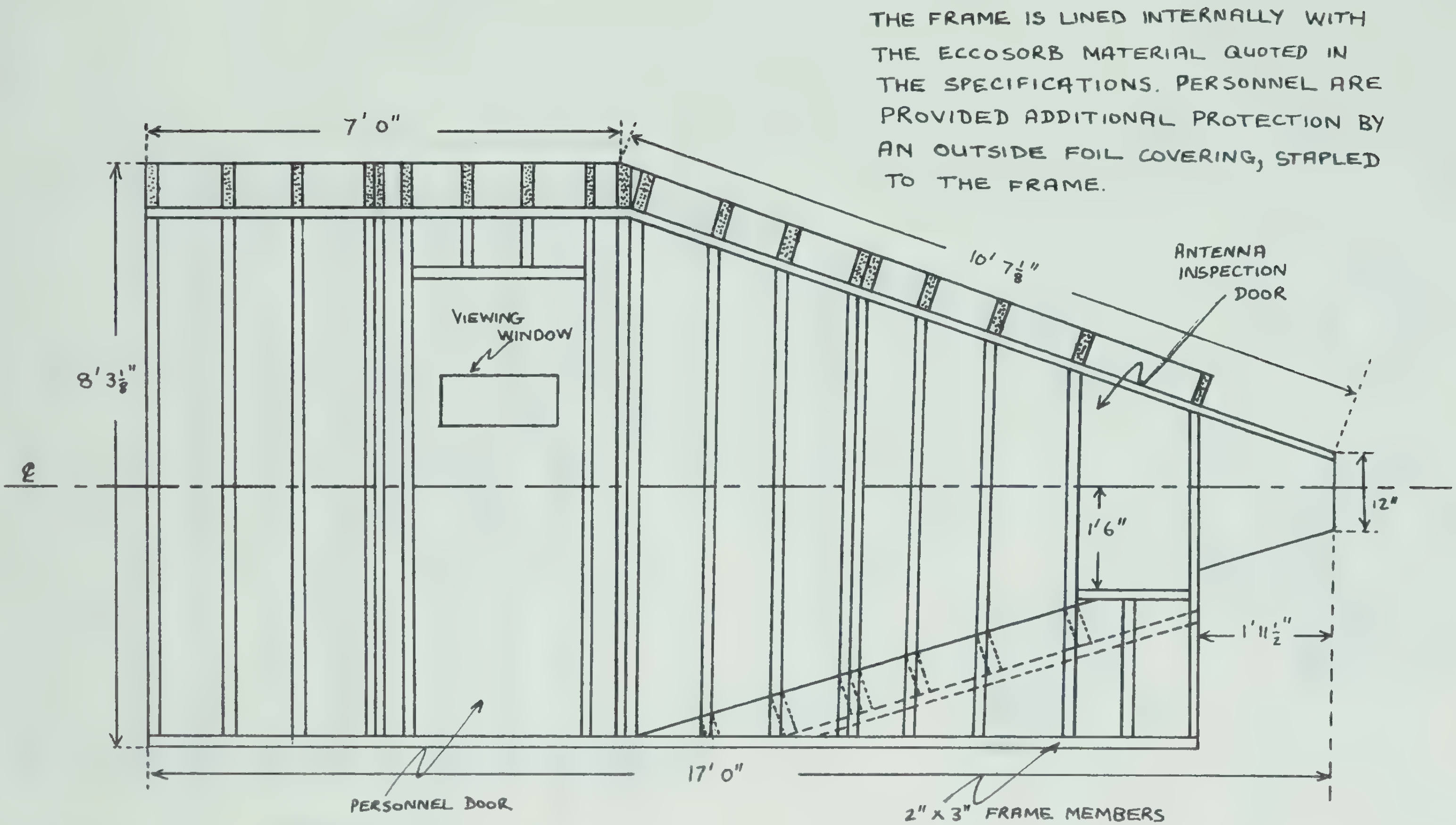
APPENDIX 6

DESIGN OF AN ANECHOIC CHAMBER

#1 refers to the chamber with the tighter measurement specifications.

		#1	#2
Length (ID)		16 ft.	16 ft.
Width (ID)		8 ft.	6½ ft.
Height (ID)		8 ft.	6½ ft.
Chamber design		tapered	tapered
Path length		10-12 ft.	10-12 ft.
Quiet zone size		D = 4 ft.	D = 3 ft.
Guaranteed minimum measurement accuracy	1Ghz	±0.5 dB	±1.0 dB
	3Ghz	±0.2 dB	±0.5 dB
	10Ghz	±0.1 dB	±0.2 dB
Illuminating antennas		standard gain horns	standard gain horns
Eccosorb Absorbers	back wall	VHP-8	VHP-4
	sides, floor, ceiling	WG-2	WG-2
	work regions walkway	FRL-340	FRL-340
	taper	WG-2	WG-2

Diagrams of the structure of the framework of a #2 chamber are given in Figs. 13 and 14. The outside of the chamber is covered with builder's foil, the inside being lined with Eccosorb materials noted above.



THE FRAME IS LINED INTERNALLY WITH THE ECCOSORB MATERIAL QUOTED IN THE SPECIFICATIONS. PERSONNEL ARE PROVIDED ADDITIONAL PROTECTION BY AN OUTSIDE FOIL COVERING, STAPLED TO THE FRAME.

SCALE: 3/8" = 1 FT.

FIG. 16 SIDE VIEW OF ANECHOIC CHAMBER

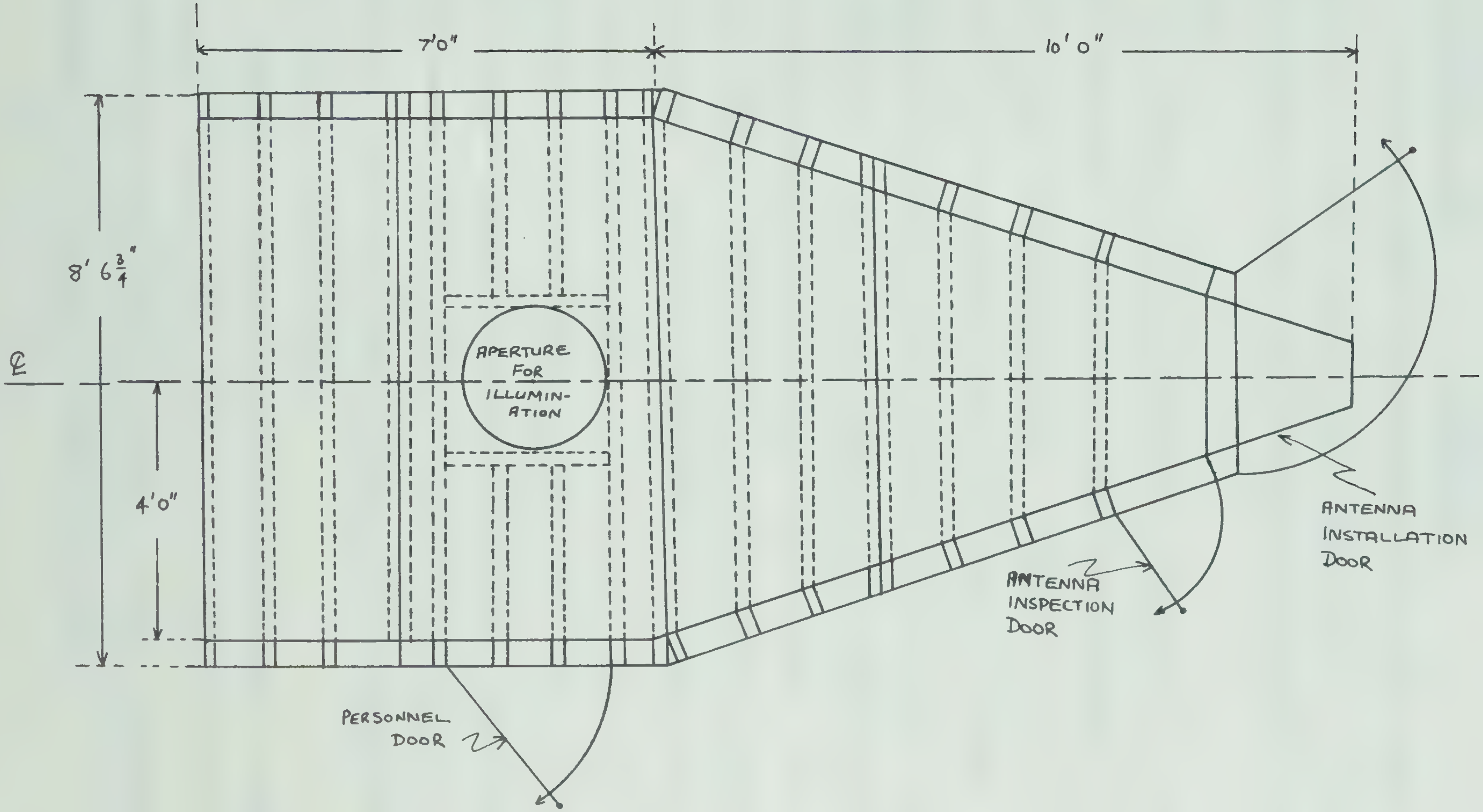


FIG. 17 TOP VIEW OF ANECHOIC CHAMBER

USE OF STATISTICAL DATA APPENDIX 7

The data obtained in the experiment have been analyzed using the t-test as explained in Chap. 9 under table 7. Several important factors affecting the probability levels should be further explained.

On examination of the experimental procedure, it is seen that the four samples as plated for control and experiment in each run are not always wholly independent. Generally, a 0.5 ml sample was taken, 0.1 ml thus being released on to each of 4 plates. Since the samples are measured by means of marks on the pipette, it is conceivable that in one case the liquid may stop slightly short of a mark, and in another case, it may go slightly beyond. This could result in a slightly larger sample. In a similar way, a small sample could be obtained. Over 4 such measurements, an initial large sample would result in subsequent smaller ones and vice versa, and so the samples would not be independent. In cases where only 0.4 mls were drawn up, as often happened, these arguments apply even more strongly as the 4th sample would be smaller or larger depending on the other 3. Only in the rather rare cases in which 4 0.1 ml samples were taken is there no interdependence.

Because of the above factors, it is difficult to decide how many degrees of freedom are to be used in estimating the probability of a deviation of plaque counts being due to chance. In normal circumstances, the two sets of 4 samples would give:

$$n_1 + n_2 - 2 = 4 + 4 - 2 = 6 \text{ degrees of freedom}$$

However, in view of the dependence of the samples on one another, 5 degrees of freedom would probably be in order and 4 would probably err on the cautious side. In this particular instance, it was decided to use 4 degrees of freedom with the understanding that the results are almost certainly an overestimate of the probability of deviations being due to chance.

Analysis using the t-test is limited in that it applies to individual experiments rather than to the set of data as a whole. It would be instructive to perform a test which would indicate the overall significance of the experiments. For this reason, the results were further analyzed using the χ^2 (chi-squared) test, for combination of probabilities from tests of significance. This test is due to Fisher and is fully described in (59).

If the results of the experiments were due to chance, one would expect a distribution of the form shown in fig. 18. Using the results of the experiments performed a curve similar to that of fig. 19 was obtained. Since the null hypothesis states that these two curves should be the same, use of the above-mentioned χ^2 test gives the probability that the difference between the curves is due to chance.

In this test, two degrees of freedom are attributed to each value and so:

$$\log_e(\text{Probability}) = -\frac{1}{2}\chi^2$$

$$\therefore \chi^2 = -2\log_e(\text{probability})$$

The χ^2 for each experiment is found in this manner and then all these χ^2 are added together.

NB. CURVES CALCULATED FOR DATA AS A WHOLE

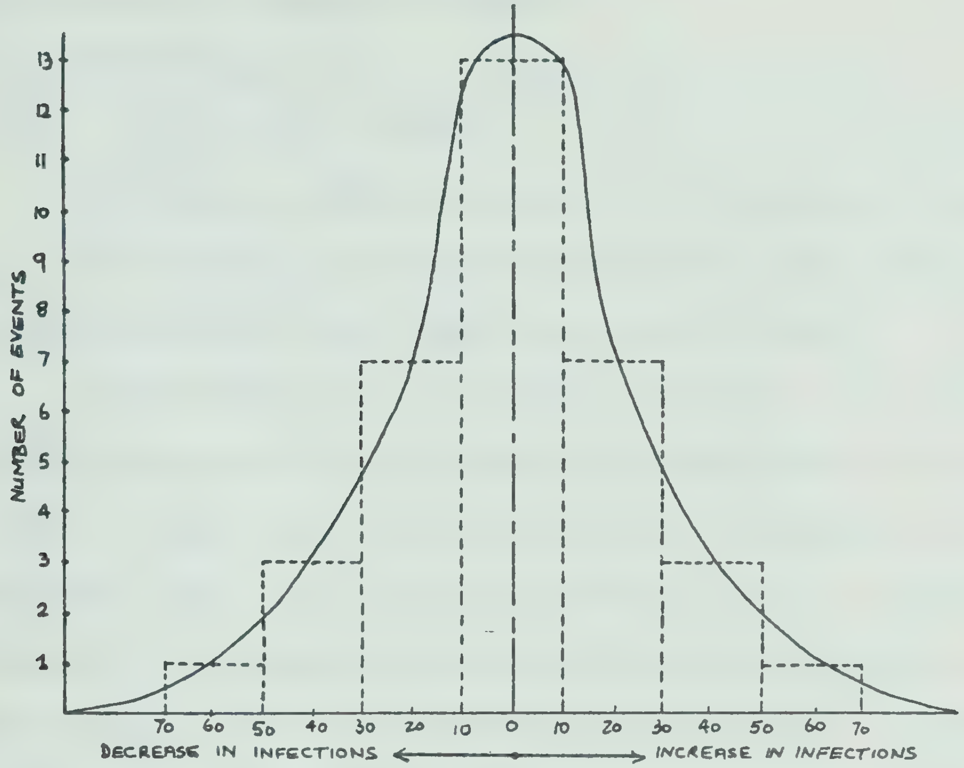


FIG. 18. EXPECTED FREQUENCY DISTRIBUTION WITHOUT RADIATION

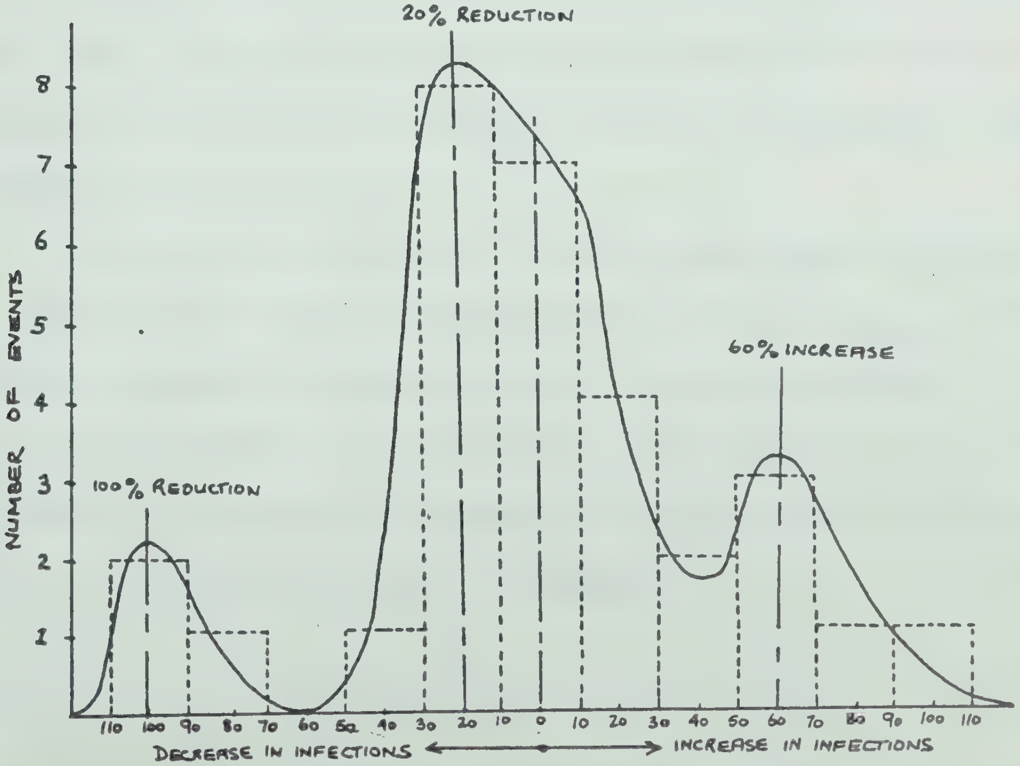


FIG 19. FREQUENCY DISTRIBUTION WITH RADIATION

In this particular case, there are 35 experiments in tables 7 and 8, giving 70 degrees of freedom.

Calculating $\sum \chi^2$

$$\chi^2_{[70]} = \sum \chi^2 = 277.74$$

Referring to tables, we see that for 70 degrees of freedom, $\chi^2_{[70]} = 112.32$ gives a probability of 0.001 and therefore the probability obtained above is many orders less than this.

Several important points should be made here. (i) In assigning probabilities in the t-test, a minimum probability of 0.001 was used, though in many experiments the actual probability was considerably less. (ii) In those experiments where the probability was higher, it was simply stated as being less than some value (see tables 7 and 8). The actual probability in these cases is therefore less than that used above. (iii) By its very nature, the χ^2 test ignores the fact that there may be increases or decreases in infection rate. (iv) As previously pointed out, the probabilities as found using the t-test are further increased owing to the choice of 4 degrees of freedom rather than 5 or 6.

Since exact calculation of t in the above cases is tedious, the maximum values from tables were used in calculating $\chi^2_{[70]}$ and so this value is less than the true value. The above calculated probability therefore errs considerably on the large side, and so there is further justification for stating that microwave radiation has an effect on the biological system in question.

APPENDIX 8

COMPLETE RESULTS

ALL SCAVENGING IN THESE EXPERIMENTS USED E. COLI K12λ

FOOTNOTE #	①	②	③	④ *	⑤					⑥	⑦	⑧	⑪	⑫
	DATE ON WHICH THE EXPERIMENT WAS PERFORMED	No. OF PHAGE x10⁷ AT TIME OF RADIATION	POWER DENSITY AND PULSE WAVEFORM	CARRIER FREQUENCY, MHZ	PLATE #1	PLATE #2	PLATE #3	PLATE #4	TOTAL OF PLAQUE COUNTS ON ALL FOUR PLATES	MEAN PLAQUE COUNT OF 4 PLATES	% AGE CHANGE FROM CONTROL	VALUE OF t IN STATISTICAL t-TEST	TITRE ADJUSTED TO THE SAME RANGE AS CONT.	SIGNIFICANCE LEVEL: NS MEANS NON-SIGNIFICANT
CONT. 6a ⑨	23/12	(4·1)	5·6	12·2 *	3	9	3	8	23	5·8	-94·9	22·0	APPROX 20·5	<·001
EXP. 6a ⑨		1·1	105/30		0	0	0	1	1	0·3				
CONT. 6b ⑨	23/12	(4·1)	4·4	12·2 *	3	9	3	8	23	5·8	-65·5	6·62	APPROX. 20·5	<·01
EXP. 6b ⑨		1·1	70/65		3	3	1	1	8	2·0				
CONT. 6c ⑨	23/12	(4·1)	3·6	12·2 *	3	9	3	8	23	5·8	-34·5	1·60	APPROX. 20·5	<0·1
EXP. 6c ⑨		1·1	25/110		3	1	4	7	15	3·8				
CONT. 9a	14/1	(4·6x10^{-4})	5·6	2426	11	4	10	11	36	9·0	-16·7	0·90	APPROX 115·0	<·5
EXP. 9a		(1·2 x10^{-5})	105/30		4	7	12	7	30	7·5				
CONT. 9b	14/1	(5·75)	5·6	2426	15	31	17	21	84	21·0	+14·3	2·78	APPROX 19·2	=·05
EXP. 9b		6·0	105/30		23	27	24	22	96	24·0				
CONT. 9c	14/1	(5·75)	5·6	2426	13	9	14	3	39	9·8	+73·5	4·28	APPROX. 19·2	<·02
EXP. 9c		6·0	105/30		18	13	21	16	68	17·0				
CONT. 10a ⑩	25/1	(1·44)	5·6	2422	0	1	0	-	1	0·3	0	0	APPROX 7·2	NS.
EXP. 10a ⑩		6·9	105/30		0	0	1	-	1	0·3				
CONT. 10b ⑩	25/1	(1·44)	5·6	2422	2	4	4	-	10	3·3	-9·1	2·30	APPROX 7·2	<·1
EXP. 10b ⑩		6·9	105/30		4	2	3	-	9	3·0				
CONT. 11a	26/1	(0·97)	5·6	2422	51	49	62	60	222	55·5	+51·4	2·76	48·6	<·05
EXP. 11a		0·98	105/30		63	76	85	112	236	84·0				
CONT. 12a	1/2	(6)	5·6	2422	67	52	41	48	208	52·0	-33·7	6·02	APPROX. 151·0	<·01
EXP. 12a		1·8	105/30		36	42	29	31	138	34·5				
CONT. 12b	1/2	(6)	5·6	2422	51	63	40	55	209	52·3	-4·3	0·30	APPROX 151·0	<0·8
EXP. 12b		1·8	105/30		65	59	44	32	200	50·0				
CONT. 12c	1/2	(6)	5·6	2422	69	70	69	80	288	72·0	+54·9	14·5	APPROX. 151·0	<·001
EXP. 12c		1·8	105/30		25	33	34	38	130	32·5				
CONT. 13a	2/2	(22·0)	5·6	2420	601	574	628	598	2401	600·3	-20·4	23·4	APPROX. 544·0	<·001
EXP. 13a		24·0	105/30		488	480	480	463	1911	477·8				
CONT. 13b	2/2	(22·0)	5·6	2420	640	684	572	545	2441	610·3	+38·2	10·2	APPROX. 544·0	<·001
EXP. 13b		24·0	105/30		846	892	795	-	2533	844·3				
CONT. 14a	3/2	(8·2)	5·6	2420	669	684	673	746	2772	693·0	+19·5	3·46	APPROX 700·0	<·05
EXP. 14a		14·0	105/30		833	936	766	776	3311	827·8				
CONT. 15a	17/2	(0·4)	6·0	2452	129	140	159	130	558	139·5	+19·2	6·28	44·0	<·01
EXP. 15a		1·9	105/30		174	157	161	173	665	166·3				
CONT. 15b	17/2	(0·4)	6·0	2452	195	190	170	161	716	179·0	-19·3	16·4	44·0	<·001
EXP. 15b		1·9	105/30		150	140	145	143	578	144·5				
CONT. 15c	17/2	(0·4)	6·0	2452	183	199	213	168	763	190·8	-26·5	17·3	44·0	<·001
EXP. 15c		1·9	105/30		133	139	147	142	561	140·3				

TABLE 9 RESULTS OF EXPERIMENTS IN ANTENNA FIELD

NOTES:

1) "Date experiment performed". All dates are 1972 except experiment 6 which was 1971.
2) "No. of phage x 10^7 at radiation". This refers to the actual number of phage present at the time that the radiation was applied. For instance, there were 6.0 x 10^7 phage present at radiation in experiment 9b. It seems that significant experiments only occurred when this figure was between 1 and 4 x 10^7. It should be pointed out that this is a number of phage, not a concentration. Further, this number was calculated by working back from the final number of plaques, and is therefore probably a little low since there is never 100% infection of bacteria by phage. The values in brackets are calculated on a basis of the concentrations as estimated from the concentration vs. time graph. They are therefore not necessarily accurate but provide a check on the other values presented, these other values being the ones used to plot the percentage change vs. concentration graph.
3) "Power and pulse waveform". This column is best explained by example. In experiment 6b, for instance, the figures 5.6, and 70/65 appear. The 5.6 refers to the measured power density in front of the waveguide horn, and is in mW/cm^2. It merely infers an order of magnitude of power density since perturbations by the sample and sample holder would cause some variation. The figures 70/65 refer to pulse shape, indicating that the microwave radiation is "on" for 70μsecs and "off" for 65μsecs.
4) "Carrier frequency, Mhz". In the case of experiment 6, the measurement is actually the wavelength in cms, ie. 12.2cms, since at this time, measurement was made with a slotted line. In all the other experiments, however, a calibrated co-axial cavity was used to make the measurements.
5) "Plates, 1 through 4". In most cases, 4 plates were poured from each of the final diluted control and experiment samples, "experiment" referring to the radiated sample. Usually, a 1.0ml pipette was used, 0.5ml of the sample being drawn up, and 0.1ml being deposited on each of 4 plates, the last 0.1ml being discarded. Thus, the 4 figures presented are somewhat dependent on each other, a slightly large sample on one plate resulting in a slightly small one on another. The extent to which these figures agree is therefore an indication of pipetteing accuracy and usually 10% variation is quite acceptable. Pipetting technique is seen to improve with later experiments. Of course, small counts (<50) probably give a somewhat erroneous indication. Occasionally, plates were prepared using a 0.1ml pipette for each sample, and though this probably gives a more accurate picture of what is present in the final solution, it is time consuming, and this is critical as previously explained.

6) "Mean of 4." Usually, this is the mean of 4 plates, but sometimes one or more plates were spoiled for some reason, and these were rejected, the mean then being taken of 2 or 3 as the case may be.

7) "Percentage change from control." This is self-explanatory except for the fact that "+" indicates an increase from control to experiment, and "-" a decrease.

8) "Statistic t test." This a statistical test performed in order to determine the significance of a set of data. The formulae employed are as follows (5).

$$t = \frac{\sqrt{n}.\,|M-m|}{s}$$

where n = no. of sample measurements.
m = mean of sample measurements.
M = mean of control.
s = standard deviation.

Having determined a value of t, reference is made to a t table which lists values of t for different numbers of samples, and for different probabilities of no significant difference between m and M. In this particular example, the probability is chosen at a level of .01 or 1%.* (See Appendix 7).

9) Experiment 6 was the first successful experiment performed. In this case, the pulse lengths were varied as indicated, and the data appear to indicate a definite dependence of inhibition on pulse length. The data are very significant on their own, but when regression analysis is applied, an even greater significance is apparent. On the basis of this experiment, it was decided to use a power density of 5.6mW/cm (nominal) and a pulse ratio of 105µsecs. "on" and 30µsecs. "off" in subsequent experiments, since this gave the greatest effect. Other power levels and pulse ratios were employed on a few occasions and though data are sparse, little effect was noted. Two factors regarding experiment 6 render it less significant. The first is that the number of plaques on the control are fairly small, (5.8 mean), and the second is that there is only one control for the three radiated samples. In subsequent experiments each radiated sample had its own control, and this is far more satisfactory.

10) Experiment 10 is simply presented for its completeness, and its results have little significance.

11) "Adjusted titre." See note (11) under table 8.

12) "Significance level." The figures in this column refer to the chance of obtaining the change from control to experiment simply as a result of random sampling. The significance levels are found from a table on page 162 of (5).

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*This level is chosen as it is a standard though arbitrary level, defined as "Difference almost certainly significant" (58).

POWER DENSITY IN ALL CASES WAS 7MW/CM², AND PULSE RATIO WAS 105/30 (SEE NOTE 3 UNDER TABLE 7)

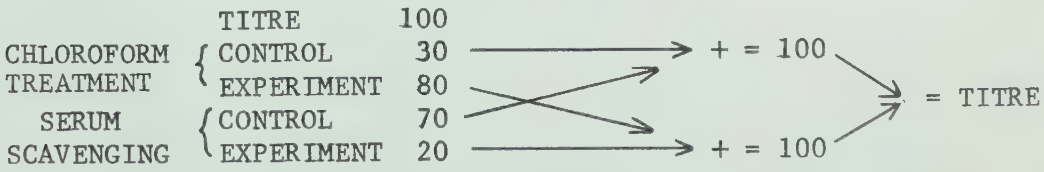
FOOTNOTE #	①	②	③		④					⑤	⑥	⑦	⑪	⑬
	DATE ON WHICH THE EXPERIMENT WAS PERFORMED	NO. OF PHAGE $\times 10^7$ AT TIME OF RADIATION	TYPE OF SCAVENGER EMPLOYED	CARRIER FREQUENCY MHZ.	PLATE # 1	PLATE # 2	PLATE # 3	PLATE # 4	TOTAL OF PLAQUE COUNTS ON ALL FOUR PLATES	MEAN PLAQUE COUNT OF FOUR PLATES	% CHANGE FROM CONTROL	VALUE OF t IN STATISTICAL t-TEST	TITRE, ADJUSTED TO THE SAME RANGE AS CONT.	SIGNIFICANCE LEVEL: NS MEANS NON-SIGNIFICANT
CONT. 16a	1/3	(2·83)	K12λ	2456	468	401	545	440	1854	463·5	-28·0	29·0	APPROX. 282·5	<·001
EXP. 16a		4·4			342	328	339	323	1332	333·0				
CONT. 18a	10/3	(4·4)	K12λ	2460	647	836	675	785	2943	735·8	+1·9	0·70	444	<0·6
EXP. 18a		8·2			797	721	770	712	3000	750·0				
CONT. 18b	10/3	(4·4)	K12λ	2460	614	441	602	603	2260	565·0	-13·5	2·40	444	<·1
EXP. 18b		8·2			477	473	583	422	1955	488·8				
CONT. 18c	10/3	(4·4)	K12λ	2460	701	796	737	745	2979	744·8	+26·3	2·92	444	<·0·05
EXP. 18c		8·2			1000	740	1000	1021	3761	940·3				
CONT. 18d	10/3	(4·4)	K12λ	2460	815	825	823	832	3295	823·8	-18·6	6·98	444	<·01
EXP. 18d		8·2			715	703	626	637	2681	670·3				
CONT. 19a	13/3	(4·2)	KCN,	2460	638	593	631	620	2482	620·5	-7·4	4·14	423	<·02
EXP. 19a		6·1	K12λ		606	555	573	565	2299	574·8				
CONT. 19b	13/3	(4·2)	KCN,	2460	660	655	629	711	2655	663·8	+1·1	0·18	423	<·9
EXP. 19b		6·1	K12λ		590	662	719	712	2683	670·8				
CONT. 20a	17/3	3·4	KCN,	2460	19	30	12	17	78	19·5	+184	3·32	170	<·05
EXP. 20a			K12λ		52	64	27	78	221	55·3				
CONT. 20b	17/3	3·4	KCN,	2460	82	73	82	87	324	81·0	+34·3	3·38	170	<·05
EXP. 20b			K12λ		94	107	132	102	435	108·8				
CONT. 20c	17/3	3·4	KCN,	2460	128	132	137	148	543	135·8	-85·6	33·0	170	<·001
EXP. 20c			K12λ		18	12	19	29	78	19·5				
CONT. 21a	21/3	1·0	KCN	2460	5	3	3	6	17	4·3	-95·7	38·4	100	<·001
EXP. 21a			$CHCl_3$		104	103	96	–	303	101				
CONT. 22a	23/3	0·7	KCN	2460	23	38	27	32	120	30	–	1·24	71	<·3
EXP. 22a			$CHCl_3$		15	28	16	36	95	23·8				
CONT. 22b	23/3	0·7	KCN,	2460	134	138	167	148	587	146·8	+55·0	4	71	<·02
EXP. 22b			ANTI-φ SERUM		198	255	269	188	910	227·5				
CONT. 23a	24/3	1·7	KCN,	2460	254	213	288	263	1018	254·5	–	2·14	173	<·1
EXP. 23a			$CHCl_3$		302	275	259	264	1100	275·0				
CONT. 23b	24/3	1·7	KCN,	2460	318	342	346	343	1349	337·3	-8·0	4·32	173	<·05
EXP. 23b			ANTI-φ SERUM		309	320	319	293	1241	310·3				
CONT. 24a	28/3	0·13	KCN,	2460	10	7	7	12	36	9	–	11·44	14	<·001
EXP. 24a			$CHCl_3$		3	2	0	2	7	1·8				
CONT. 24b	28/3	0·13	KCN,	2460	179	155	135	160	629	157·3	-8·3	1·24	14	<·3
EXP. 24b			ANTI-φ SERUM		163	142	116	156	577	144·3				
CONT. 25a	13/4	7·4	KCN,	2460	4	6	5	9	24	6	0·68	7·46	737	<·01
EXP. 25a			$CHCl_3$		3	1	1	0	5	1·3				

TABLE 10. RESULTS OF EXPERIMENTS IN WAVEGUIDE

NOTES:

1) "Date experiment performed". In all cases, these dates refer to 1972.
2) "No. of phage x 10^7 radiation". See note (2) under table 7.
3) "Type of scavenger employed". This point is more fully illuminated in chapter 7. It should be explained here however, that K12(λ) refers to the scavenging bacteria, E.coli K12(λ); $CHCl_3$ refers to those experiments in which all bacteria (infected and uninfected) are destroyed with chloroform; anti-ϕ serum refers to those experiments in which the unadsorbed phage is neutralized by means of the anti-phage serum. Also included in this column is an indication of those experiments in which KCN inhibitor was employed. The purpose of this inhibitor is described in chapter 7.
4) "Plates 1 through 4". See note 5 under table 7.
5) "Mean of 4". See note 6 under table 7.
6) "% change from control". See note 7 under table 7.
7) "Statistical t test". See note 8 under table 7.
8) Experiment 18 was performed as a check on technique. The author did sections 18a and 18b and an independent investigator (Vera Perekóvic) did sections 18c and 18d.
9) The experiment noted 19 was performed by the author and a second independent investigator (Dr. Hodgetts) working in conjunction, this time as a check on experimental protocol and technique.
10) The experiments bracketed here were all performed with both $CHCl_3$ and Anti-ϕ Serum in the hope that complementary results could be obtained. Along with these, as with most other experiments, titre experiments were performed. An example best illustrates what should happen.

Consider an experiment in which the phage is diluted to give 100 plaques. Assume further that there is a 70% adsorption in the experimental sample, during the radiation period. We then have:



Thus we see that by adding the two controls, we should get the same number of plaques as the titre, and similarly for the two experimental samples.

Complete details as to the mechanism of the scavengers is given in chapter 7, and the relevant titre measurements are in table 9. The adjusted titre value in this table should be compared with the controls in table 8.

Unfortunately, the results of the experiments in question are unsatisfactory in this respect.

11) "Adjusted titre". This is a value of the virus titre which has been adjusted to enable direct comparison with the control and experimental results. ie. It is an indication of what the control should be, assuming 100% adsorption of phage by bacteria. Adjustment is necessary since the dilutions were not always the same. As can be seen, the control is sometimes higher than the titre, and this suggests premature bursting. There may well be another reason for this, however, since premature bursting is extremely unlikely in experiments 19 to 24.

It should be noted that some of the titre values have been calculated from the graph plotted along with the titre results, and so there may be some deviation form the actual value on the day of the experiment. Such calculation was necessary since titre experiments were not performed each time. The word "approx". identifies such calculated values of titre.

12) In experiments where chloroform is employed to remove all bacteria (infected and uninfected), the relative plaque counts of control and experiment samples would be reversed as compared to those experiments using other methods of scavenging. Thus the control count should be lower than the experiment count if infections of bacteria by phage are reduced.

13) "Significance level". See note (12) under table 7.

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